Handbook of vitamin D in human health

Prevention, treatment and toxicity

edited by:
Ronald Ross Watson

Handbook of vitamin D in human health

Handbook of

vitamin D in human health

Prevention, treatment and toxicity

Edited by:
Ronald Ross Watson

Human Health Handbooks no. 4

ISSN 2212-375X

Wageningen Academic
P u b l i s h e r s

ISBN: 978-90-8686-210-8
e-ISBN: 978-90-8686-765-3
DOI: 10.3920/978-90-8686-765-3

First published, 2013

Table of contents

Overview of key vitamin D modified conditions

Vitamin D and musculoskeletal health

Vitamin D and chronic disease

Vitamin D and infectious disease

Vitamin D and cancer

Vitamin D in chronic diseases

Women and children

Index

Overview of key vitamin D modified conditions

Key facts

- Historically, vitamin D deficiency was defined by the clinical disease of rickets, which was nearly eradicated through vitamin D fortification.
- Presently, defining optimal vitamin D status based on concentrations of the serum 25-hydroxyvitamin D (25(OH)D) metabolite is controversial. The functional consequences of a given 25(OH)D concentration are highly variable within the population, but up to one billion people worldwide may have less than optimal vitamin D status.
- Vitamin D is actually a hormone, not a true vitamin. It can be ingested (naturally occurring in fatty fish, fortified in some foods such as milk or in supplement form) or dermally synthesized with exposure to ultraviolet light.
- Observational studies have demonstrated relationships between vitamin D status and infectious, immune, metabolic, degenerative, and neoplastic diseases, but the beneficial effect of vitamin D in these conditions requires confirmation with randomized controlled trials.
- Identifying markers of the diverse functional effects of vitamin D will permit an individualized approach to vitamin D supplementation, based on health risks.

Summary points

- Vitamin D deficiency causes the clinical disease of nutritional rickets, which causes softening and bending of the bones.
- The incidence of vitamin D-deficiency rickets declined dramatically with fortification of milk and foods with vitamin D.
- A recent worldwide resurgence of rickets has been recognized, possibly related to reduced intake of vitamin D, breastfeeding practices, increasing prevalence of obesity, sun avoidance and sunscreen use, and air pollution.
- Vitamin D status is currently defined by the serum 25(OH)D concentration, which is a marker of storage rather than function. Laboratory assays for measurement of 25(OH)D vary widely.
- There is no universally accepted serum-based definition of vitamin D deficiency, but nutritional rickets occurs at very low 25(OH)D concentrations.
- The Institute of Medicine recommended 600 IU of vitamin D daily for most adults. However, several intermittent dosing regimens have been proposed for skeletal and non-skeletal health benefits.
- The health benefits of sufficient vitamin D may extend far beyond bone health. Observational studies suggest that vitamin D may benefit immunity, cardiovascular health, several cancers, pain, and muscle strength.
- Wide variation in individual responses to vitamin D exists. As personalized medicine becomes more of a reality, and the non-skeletal benefits of vitamin D are better defined, recommended vitamin D intake will need to be personalized.

1. Vitamin D deficiency in the 21st century: an overview

S.S. Oberhelman and T.D. Thacher
Department of Family Medicine, Mayo Clinic, 200 1st St SW, Rochester, MN 55905, USA;
thacher.thomas@mayo.edu

Abstract

Vitamin D deficiency in the past was defined by the clinical recognition of nutritional rickets, a disease nearly eradicated by vitamin D fortification. Today, the definition of vitamin D deficiency is controversial, and is based on serum concentrations of 25-hydroxyvitamin D (25(OH)D), a marker of storage rather than of function. The level of 25(OH)D that is optimal for bone health may differ from that required for non-skeletal benefits. Observational studies suggest wide-ranging effects of vitamin D status on metabolic, immunologic, infectious, cardiovascular, neoplastic, and degenerative disorders. There is likely wide individual variation in the functional implications of a given level of 25(OH)D. As randomized controlled trials confirm the benefits of vitamin D in non-skeletal diseases, the recommended intake of vitamin D and optimal 25(OH)D concentration may need to be adjusted to account for these outcomes. The future will bring an individualized approach to assessing vitamin D status and needs. Even when the factors known to influence 25(OH)D concentrations are accounted for, most of the individual variation of 25(OH)D values is difficult to explain. This variation is partially explained by genotypic variants of the vitamin D binding protein, vitamin D receptor, and the hydroxylase enzymes of the vitamin D metabolic pathway. With more widespread use of genome-wide analysis, it may be possible to personalize an individual's vitamin D requirement, particularly in view of other disease risk factors. Measurement of the physiological effects of vitamin D that relate to bone metabolism, the immune system, or other effectors of disease could allow tailoring of the dose of vitamin D to attain maximal physiological benefit. Additional markers of the functional response to vitamin D at the individual level may be available in the future. Vitamin D has exciting potential, and a flurry of research activity is now underway to explore its potential benefits.

Keywords: rickets, hypovitaminosis D, public health, deficiency, personalized medicine

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_1, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
CDC	Center for Disease Control
IU	International unit
NHANES	National Health and Nutrition Examination Survey
UV	Ultraviolet

1.1 Vitamin D: yesterday

To fully appreciate the current and future implications of vitamin D insufficiency, we must explore its historical context. Historically vitamin D deficiency was defined by the clinical recognition of rickets, the childhood disease of impaired skeletal development, or osteomalacia in adults. The first descriptions of clinical manifestations of rickets appeared in ancient times. Second century Roman physician Soranus published a text in which he recommended swaddling to prevent deformities common in city dwellers (Foote, 1927; Rajakumar, 2003). Modern scientists examined a pediatric skull and radius from pre-Columbian Peru and found both consistent with rickets. In the 1920s John Foote examined fifteenth century paintings from the Netherlands and Germany and concluded that the infant subjects showed evidence of rickets (Foote, 1927).

The term 'rickets' first appeared in 1634 when listed as a cause of death in London's Annual Bill of Mortality (O'Riordan, 2006). Four publications regarding rickets appeared in the mid-seventeenth century: Daniel Whistler's doctoral defense thesis (O'Riordan, 2006; Smerdon, 1950), Arnold Boote's chapter in his book 'Medical Observations on Neglected Ailments', Francis Glisson' book which described findings seen on post mortem examinations and John Mayow's book. For the next century and a half, little occurred in regards to rickets in the medical community (O'Riordan, 2006).

Investigators began studying the potential relationships between rickets and both cod liver oil and sunlight in the early 19th century. Unpublished rumors and subsequent case reports began spilling out of Holland, Germany and France: The Society of Science and Arts of Utrecht described the therapeutic properties of cod liver oil in 1822 (Guy, 1923); German physician Schutte published a 25 year case history of his successes in curing rickets with cod liver oil in children in 1824 (Guy, 1923); and French physician Bretonneau reported successfully treating a 15 month old suffering from rickets in 1827 (Holick, 1994; Rajakumar, 2003).

Scientists also observed a relationship with sunlight. In 1822 Sniadecki noted a higher incidence of rickets in children living in Warsaw than children living in surrounding rural communities; he blamed inferior sunlight exposure within the city as the causative factor (Holick, 1994; 2003). In 1838 Jules Guerin experimented on puppies: he weaned puppies to a dark basement and fed them raw meat while the littermates remained in a normal environment. He reported the basement

puppies 'looked sad', vomited, and developed misshapen limbs and other signs of rickets while the other pups had no such signs (Mayer, 1957). Hugh Owen Thomas treated rachitic children with sunlight exposure on hospital balconies in Wales in 1878 (Chesney, 2012). While on a medical mission in Japan, Theobald Palm noted the absence of rickets. He collected prevalence data from worldwide missionaries and noted that areas of temperate latitude or heavier air pollution had more cases or rickets but that even filthy areas of the jungle did not; he concluded sunlight as the protective factor. August Hirsch and the Investigation Committee on the British Medical Association developed similar maps but blamed overcrowding and air/soil conditions (Chesney, 2012; Holick, 2003). Finally, John Bland-Sutton observed monkeys and lions in glass enclosures at the London Zoo and noted development of rickets within months of the animals' arrivals or births. However, he noted that lion cubs receiving cod liver oil and crushed bones to their usual lean-meat diet recovered from their rickets-features. He hypothesized dietary fat cured rachitic features (Findlay, 1908; Mayer, 1957; O'Riordan, 1997; Park, 1923; Rajakumar, 2003).

By the turn of the 20th century, speculations regarding the etiology of rickets varied: syphilis, scurvy, genetics, acidosis causing bony decalcification, strep infection, confinement/exercise deprivation, over-eating, or disorders of the thymus, thyroid, adrenal, parathyroid, or carotid (Cheadle, 1988; Findlay, 1908; Park, 1923). Studies of scurvy, beri-beri, and pellagra led to the discovery of 'vital substances' (later 'vitamins') which, in deficiency, led to disease. (Chesney, 2012) At this same time, rickets had become epidemic in the US and Europe: autopsy studies identified rickets in 80-96% childhood deaths (Holick, 1994; Park, 1923; Rajakumar, 2003), oral exams in London school children saw rickets in the teeth of 80% (Mellanby, 1919), and estimates of children living in industrialized cities exceeded 90% (Holick, 2003). Women with pelvises flattened by the affects of childhood rickets began requiring Cesarean births (Holick, 2003).

The early 1900s represented a turning point in rickets research. In terms of light-related work, Buchholz, Schmorl, Hess, Lundagen and Raczynski all noted seasonal variations in the incidence of rickets (Chesney, 2012; Park, 1923). German pediatrician Kurt Huldchinsky treated over 100 cases of childhood rickets with quartz mercury-vapor lamp UVB irradiation and then noted that UVB exposure to a single limb produced X-ray evidence of full skeletal healing (Chesney, 2012; Holick, 1994; 2003; Park, 1923; Rajakumar, 2003). Harriett Chick and her colleagues treated children with rickets at a Vienna hospital after World War I. They treated children with nothing, mercury-vapor lamp UVB exposure, sunlight, cod liver oil, or a combination of UVB and cod liver oil. They found several interesting results: healing rates depended on amount of UVB exposure; partial body UVB exposure produced full body healing; no new cases of rickets developed during the summer months in any group; and most rapid healing occurred in children receiving both UVB exposure and cod liver oil (Chick *et al.*, 1922; O'Riordan, 1997). Additionally Hess studied epiphyses calcification on radiography and published that irregular sunlight exposure, in the absence of diet fortification, improved rickets (Hess and Mildred, 1925b; Holick, 1994).

From a diet-perspective, English physician Edward Mellanby varied the diets of puppies and noted the protective factor of cod liver oil, butter and whole milk. He postulated poor intake of 'an antirachitic factor which is either fat-soluble A or has a somewhat similar distribution to the

fat soluble A' as the cause of rickets (Mellanby, 1919). Elmer McCollum, John Holand, Edward Park and Paul Shipley induced rickets in rats and then healed those manifestations with cod liver oil. They then oxidized cod liver oil to isolate the anti-xerophthalmic and anti-rachitic properties and in 1922 they deduced that the two factors were distinct (McCollum *et al.*, 1921, 1922). They postulated that this factor was a vitamin and, as the fourth vitamin discovered, named it vitamin D (Holick, 1994; McCollum *et al.*, 1921, 1922; Rajakumar, 2003). Two research groups (Hess and Weinstock, Steenbock and Black) noted that previously anti-rachitic-deficient foods would gain anti-rachitic activity when irradiated with UV light (Hess, 1924; Hess and Mildred, 1925a; Holick, 1994; Steenbock, 1924). University of Wisconsin's Harry Steenbock not only published his findings regarding the ability to impart anti-rachitic factor to rat feed via UV irradiation, but patented the process 'to protect the interest of the public in the possible commercial use of these findings' (Schneider, 1973; Steenbock, 1924; Steenbock and Black, 1924; Steenbock and Nelson, 1924). The University created a board of trustees to manage the patent. Quaker Oats attempted to purchase exclusive rights, but Steenbock and the board refused. They sold licenses to Quaker Oats for cereal fortification and to a pharmaceutical agency to develop Viosterol (medicinal vitamin D supplementation). They refused to sell licenses to manufacturers of chewing gum, tobacco, lipstick, beer or soft drinks, deciding that only healthy foods should receive fortification. With dairy-farmer roots, Steenbock also developed a process to fortify milk. Laws at the time prohibited any additives to milk; three manufacturers developed equipment to irradiate flowing films of milk. (Wisconsin Alumni Research Foundation)[1] Initially milk was supplemented to 400 IU of vitamin D per liter, the same amount of vitamin D in one teaspoon of cod liver oil (Chesney, 2012). This process of fortification revolutionized the prevention of rickets (Wisconsin Alumni Research Foundation).

The next boom in vitamin D research started in the 1960s. Researchers at the University of Wisconsin isolated a metabolite chromatographically-distinguishable from vitamin D3, subsequently determined its structure as 25-hydroxyvitamin D (25(OH)D) and discovered it was produced in the liver (Blunt *et al.*, 1968; Norman *et al.*, 1964; Ponchon and DeLuca, 1969). They found a third distinct metabolite (Haussler *et al.*, 1968; Lawson *et al.*, 1969) and researchers in Cambridge, after determining production occurred in the kidney, diagramed our modern understanding of vitamin D synthesis and metabolism (Fraser and Kodicek, 1970). In 1971 three separate labs (University of Wisconsin, Cambridge and University of California) confirmed the structure of this metabolite as $1,25(OH)_2D$ (Holick *et al.*, 1971; Lawson *et al.*, 1971; Macintyre *et al.*, 1977; Norman *et al.*, 1971). Scientists re-classified vitamin D as a hormone and not a true vitamin (Holick, 1994). In 1979 Stumpf published results of ^3H nuclear localization in rats that showed vitamin D receptors in the stomach, parathyroid, gonads, brain and skin. Subsequent studies found vitamin D receptors in a plethora of other body tissues (Holick, 1994; Stumpf *et al.*, 1979). Finally, in 1980, Holick described the dermal synthesis of vitamin D (Holick *et al.*, 1980).

[1] Wisconsin Alumni Research Foundation, Steenbock and WARF's Founding. Available at: http://www.warf.org/about/index.jsp?cid=26&scid=33. Accessed 5/25/2012.

1.2 Vitamin D: today

1.2.1 Modern understanding of vitamin D metabolism

As the 19th century scientists suspected, both diet and skin exposure to UVB provide vitamin D. It occurs naturally in few foods, namely fatty, ocean dwelling fish (salmon, sardines, mackerel, tuna, and cod), shiitake mushrooms and egg yolks. It is also fortified in some cereals (100 IU/serving), milks (100 IU/227 g), orange juice (100 IU/227 g), butters (50 IU/99 g), margarines (430 IU/99 g), cheeses (100 IU/85 g) and yogurts (100 IU/227 g). (Holick, 2007) Vitamin D exists in two forms: D2 (ergocalciferol), which is produced via irradiation of yeast, and D3 (cholecalciferol), which is the form found in fatty fish and from dermal synthesis following UV exposure (Holick, 2006). As scientists deduced in the 1960s, regardless of the source, the parent compound is metabolized to 25(OH)D (calcidiol) in the liver and then further metabolized to $1,25(OH)_2D$ (calcitriol) in the kidney. The $1,25(OH)_2D$ is the active hormone that acts on vitamin D receptors throughout the body.

1.2.2 Defining vitamin D deficiency... not as easy as it sounds

Today, the definition of vitamin D deficiency has expanded from the clinical diagnosis of rickets to a definition based on the serum concentration of 25(OH)D. However, this is an imperfect marker, because it represents vitamin D supply rather than vitamin D function. The 25(OH)D metabolite has no physiologic function, and its conversion to active $1,25(OH)_2D$ is tightly regulated. Rickets or osteomalacia typically occur with serum 25(OH)D values <10 ng/ml (1.0 ng/ml = 2.5 nmol/l) (Thacher and Clarke, 2011). Optimal bone health and other non-skeletal benefits may be gained from 25(OH)D concentrations greater than those necessary to prevent rickets and osteomalacia. Currently, no universal standard definition of vitamin D deficiency exists. The most common definitions include: deficiency <20 ng/ml (<50 nmol/l), insufficiency 20-30 ng/ml (50-75 nmol/l) and sufficiency >30 ng/ml (>75 nmol/l) (Heaney and Holick, 2011; Holick, 2007), or deficiency <12 ng/ml (<30 nmol/l), insufficiency 12-20 ng/ml (30-50 nmol/l) and sufficiency >20 ng/ml (>50 nmol/l) (Institute of Medicine, 2011).

However, the clinical utility of the term 'insufficiency' is not at all clear. Because the functional effect of a given 25(OH)D value is widely variable, it is likely that the range defined as insufficiency represents adequate vitamin D status for some and deficiency for others. The requirement for vitamin D appears to be lower when the calcium intake is adequate. Definitions of insufficiency have been based on analysis of parathyroid suppression and/or calcium absorption. Some have argued that parathyroid suppression reaches a plateau at mean 25(OH)D concentrations of 28-40 ng/ml. However, flaws of these studies included substantial individual variation, lack of a clear inflection point, and exclusion of children (Heaney, 2004; Holick, 2007; Lee *et al.*, 2008; Thacher and Clarke, 2011). Calcium absorption has been reported to plateau at a mean 25(OH)D concentration of 32 ng/ml. However, additional studies fail to show a relationship between serum 25(OH)D concentrations and intestinal calcium absorption, except at concentrations below 5 ng/

ml (Heaney, 2004; Holick and Chen, 2008; Need *et al.*, 2008; Thacher and Abrams, 2010; Thacher and Clarke, 2011).

1.2.3 Return of rickets to a modern society

Following routine fortification of foods with vitamin D, the incidence of rickets declined dramatically, and it was considered rare by the 1960s. However, a recent worldwide resurgence of rickets has been recognized (Thacher *et al.*, 2006). Best estimates of national prevalence come from chart reviews and case report series since rickets is not a reportable disease (Nield *et al.*, 2006). Estimates range from 6-9 cases per million hospitalized children in the US (Centers for Disease Control and Prevention, 2001; Scanlon, 2001) or Canada (Ward *et al.*, 2007). Additionally, meta analyses of published cases found 166 cases (in 22 studies) 1986-2003 (Weisberg *et al.*, 2004) and 65 cases (in 11 studies) 1975-1985 (Cosgrove and Dietrich, 1985). The CDC has expressed concern regarding the prevalence of rickets, especially among darkly pigmented children (Scanlon, 2001).

1.2.4 Prevalence of hypovitaminosis D

Estimates of worldwide vitamin D deficiency exceed 1 billion individuals (Bell, 2011; Holick, 2007; Makariou *et al.*, 2011). In the USA, NHANES reported overall national mean serum concentrations of 25(OH)D at 30 ng/ml in 1988-1994 (Ginde *et al.*, 2009a), 24 ng/ml in 2001-2004 (Ginde *et al.*, 2009a) and 19.9 ng/ml in 2005-2006 (Forrest and Stuhldreher, 2011). International studies show mean 25(OH)D values of 8.2 ng/ml in Iranian adults (Van Schoor and Lips, 2011); 14 ng/ml in veiled and 17.2 ng/ml in non-veiled African women in Tunisia (Van Schoor and Lips, 2011); and 11-12.8 ng/ml in Middle Eastern university students living in Riyadh (Sedrani, 1984). A British study sampled 45 year old adults from a birth cohort and found winter values below 10, 16 and 20 ng/ml in 15.5, 46.6% and 87.1% respectively (Hypponen and Power, 2007).

Several studies address postmenopausal women, given their risk of osteoporosis. The prevalence of vitamin D deficiency (25(OH)D<30 ng/ml) was 50% in Thailand and Malaysia, 75% in the USA and 90% in Japan and South Korea (Dawson-Hughes *et al.*, 2010). In the UK, South Asian immigrants and Caucasian natives possessed mean serum concentrations of only 4.3 and 16.3 ng/ml respectively (Lowe *et al.*, 2010). Italian investigators found values less than 12 ng/ml and 5 ng/ml in 76% and 27% of women, respectively (Isaia *et al.*, 2003).

Children require vitamin D to establish optimal bone density during growth. Projections based on data from NHANES 2001-2004 suggested deficiency (<15 ng/ml) in 7.6 million US children and insufficiency (15-29 ng/ml) in another 50.8 million children (Kumar *et al.*, 2009). Other studies have shown deficiency (<20 ng/ml) in 48% of healthy Maine girls ages 9-11 at some point during a three year study (Sullivan *et al.*, 2005), 12.1% of healthy Boston infants and toddlers (Gordon *et al.*, 2008), and 42% of Boston adolescents (with 24.1% <15 ng/ml and 4.6% <8 ng/ml) (Gordon *et al.*, 2004). Mean serum concentrations of 11.8 ng/ml (with 35.7% below 9 ng/ml) in New Delhi children (Marwaha *et al.*, 2005), 17 ng/ml in Lebanese youth in the spring (El-Hajj

Fuleihan *et al.*, 2001), and 5 ng/ml in Chinese female adolescents (Van Schoor and Lips, 2011) have been reported.

Vitamin D deficiency also affects men. A study of community-dwelling men in several USA cities revealed a mean serum concentration of 25.1 ng/ml with 71.1% of participants below 30 ng/ml, 25.7% below 20 ng/ml and 2.9% below 10 ng/ml (Orwoll *et al.*, 2009).

Hypovitaminosis D even extends into sunny climates. A study that recruited healthy high-sun-exposure participants from a surf shop in Honolulu Hawaii found 51% of study participants' serum concentrations of 25(OH)D below 30 ng/ml (Binkley *et al.*, 2007). Similarly children in Texas showed serum levels <12, 20 and 32 ng/ml in 1%, 16% and 68% of participants, respectively (Rovner and O'Brien, 2008).

Those with sub-par health commonly possess low values. Medical inpatients at Massachusetts General Hospital were found to have a mean serum concentration of 15 ng/ml with 57% below 15 ng/ml and 22% below 8 ng/ml (Thomas *et al.*, 1998). Estimates of vitamin D deficiency in the non-institutionalized elderly range from 40 to 100% (Holick, 2007). A study of elderly individuals >98 years found undetectable serum values in 95% (Passeri *et al.*, 2003).

Finally, post partum women and their infants often have serum 25(OH)D concentrations shortly after birth below 15 ng/ml (Taha *et al.*, 1984). Prevalences of deficiency have been reported at 50% (mothers <20 ng/ml) (Lee *et al.*, 2007), 65% (Boston newborns <12 ng/ml) and 45.6% (black Pittsburgh newborns <15 ng/ml) (Rovner and O'Brien, 2008). Due to the low vitamin D content of breast milk, breastfeeding has been identified as a risk factor for vitamin D deficiency. One study found 48% of mothers and 43% of babies with 25(OH)D values <10 ng/ml (Seth *et al.*, 2009).

1.2.5 Why has this become a problem again?

Several factors may contribute to the prevalence of hypovitaminosis D and the resurgence of rickets in our modern society.

A variety of medical conditions interfere with vitamin D absorption (i.e. celiac disease, cystic fibrosis, Crohn's disease, or gastric bypass surgery), hepatic conversion or renal synthesis of vitamin D metabolites (Tsiaras and Weinstock, 2011). Medications, including anticonvulsants, glucocorticoids, antiretrovirals, and antirejection drugs can interfere with vitamin D metabolism (Holick, 2007).

The prevalence of obesity has increased throughout the world, and fat soluble vitamin D is sequestered in adipose tissue. A study evaluated serum levels of 25(OH)D in obese and ideal-weight-matched-controls either 24 hours after whole body UV irradiation or oral ingestion of 50,000 IU vitamin D2. Basal values between groups were similar, but the amount of increase in the nonobese subjects was 57% higher (15.3 versus 6.7 ng/ml) after UVB irradiation. Body mass

index was inversely correlated with serum concentrations after either UVB irradiation or oral ingestion (Wortsman *et al.*, 2000).

Several barriers exist that prevent adequate sunlight exposure. Recommendations regarding skin cancer promote sun avoidance and sunscreen use. When used properly, sunscreen with SPFs of 8 or 15 reduce cutaneous vitamin D synthesis by 97.5% and 99% respectively (Holick, 2003; Holick and Chen, 2008; Scanlon, 2001). Para-aminobenzoic acid (a sunscreen) applied to human epidermis blocked production of vitamin D with UV exposure compared with marked production of vitamin D in para-aminobenzoic acid-free epidermis samples (Matsuoka *et al.*, 1987). A study of sunscreen users and non-users with equivalent sunlight exposure revealed significantly lower serum 25(OH)D concentrations in sunscreen users (16.8 ng/ml versus 36.5 ng/ml) (Matsuoka *et al.*, 1988). Additionally, pollution impedes cutaneous vitamin D synthesis from sunlight. Black carbon from biomass and fossil fuel combustion absorbs UVB radiation from sunlight and decreases the amount that reaches the earth's surface by approximately 5% in typical urban environments (Highwood and Kinnersley, 2006) and up to 81% in the Brazilian rain forests (Mims, 1996).

The seasonal tilt of the earth can impact the amount of UVB available for cutaneous vitamin D synthesis. An appropriate zenith angle is required for UVB to penetrate the non-polluted ozone. Latitudes above 35°N and below 35°S produce too oblique of an angle during winter months for UVB penetration (Holick, 2003). This produces almost complete cessation of cutaneous vitamin D synthesis seasonally in some parts of the world: Rome (41.9°N) November through February (Tsiaras and Weinstock, 2011), Berlin (52.5°N) October through April (Tsiaras and Weinstock, 2011), Boston (42°N) November through February (Webb *et al.*, 1988), Edmonton Canada (52°N) mid-October through mid-April (Holick, 1994; Webb *et al.*, 1988).

Purdah, cultural covering, limits sunlight exposure and cutaneous vitamin D synthesis, even in the sunniest areas of the world (Holick and Chen, 2008). Window-glass blocks all UVB, eliminating any benefit for those who remain indoors (Cannell and Hollis, 2008). Melanin in the epidermis of darkly pigmented skin reduces vitamin D synthesis (Aloia, 2011). Finally, advancing age decreases cutaneous 7-dehydrocholesterol necessary for synthesis of vitamin D (Holick, 2003; Holick and Chen, 2008; Holick *et al.*, 1989; MacLaughlin and Holick, 1985).

Fortification revolutionized the world's approach to vitamin D deficiency. However, levels of fortification can vary significantly. For instance, fortified milk is advertised to contain 400 IU of vitamin D per quart. However, a study of randomly selected milk found that only 29% of the samples tested contained 320-480 IU/quart, 50% of samples contained <80% of advertised amount, 14% contained <5% advertised amount and 21% of the skim milk samples contained no detectable vitamin D (Holick, 1994; Holick *et al.*, 1992; Scanlon, 2001). A four year study in New York found that only half of sampled milk complied with label declarations (Murphy *et al.*, 2001; Yetley, 2008).

Few foods naturally contain vitamin D. Ocean-dwelling salmon contains 600-1000 IU/99 g. However, canned salmon contains 300-600 IU/99 g and farmed salmon contains only 100-250 IU/99 g (Holick, 2007).

Finally, breastfeeding has played a role. HealthyPeople 2010 set a goal for 75% of infants to breast-feed for the first six months of life (US Department of Health, 2000). Between 1988 and 1999, breastfeeding among African American mothers in North Carolina increased from 5.2% to 34.7%. During this interval several cases of rickets were identified in the same region. Breast milk has low concentrations of vitamin D; therefore, the American Academy of Pediatrics recommends universal supplementation of vitamin D to exclusively breastfed infants. However, compliance with this recommendation is poor. Data regarding supplementation of lactating mothers is somewhat new and not ready for universal recommendations (Scanlon, 2001).

1.2.6 Supplementation regimens

Significant controversy exists regarding optimal supplementation dosing; partly related to the lack of universally accepted optimal serum values. Additionally, while D2 and D3 supplements both increase in 25(OH)D values, D3 has produced slightly superior results, suggesting a higher dose of D2 needed to achieve similar serum 25(OH)D concentrations (Binkley *et al.*, 2011). Finally, the variety of dosing regimens is confusing. A review of 306 patients with vitamin D insufficiency at an Atlanta VA revealed 36 different oral vitamin D prescribing regimens. Doses ranged from 400 to 50,000 IU given twice daily to once monthly. The three most commonly utilized regimens (50,000 IU weekly ×4, then monthly ×5; 50,000 IU monthly ×6; 50,000 IU three times weekly ×6 weeks) all increased the serum 25(OH)D concentration but only achieved concentrations >30 ng/ml in 38%, 42% and 82% of subjects respectively. No toxicities were reported for any regimen (Pepper *et al.*, 2009). Each additional 100 IU (2.5 μg) increment of daily oral vitamin D3 increases serum 25(OH)D by about 1 ng/ml (0.6-1.2 ng/ml = 1.5-3 nmol/l) (Barger-Lux *et al.*, 1998; Dawson-Hughes *et al.*, 2010; Heaney, 2004).

With support from the United States and Canadian governments, the Institute of Medicine updated the Dietary Reference Intakes for calcium and vitamin D in 2011. The committee concluded that neither adequate evidence regarding the cause/effect outcomes nor the dose-response relationship currently exists for vitamin D; therefore, they made recommendations solely based on bone health. The recommended RDAs strive to achieve >20 ng/ml 25(OH)D in >97.5% of the population: 600 IU daily in ages 1-70 years (including pregnant and lactating women) and 800 IU daily in elderly (>70 years). The tolerable upper intake level was set at 4,000 IU/day for individuals >9 years. The committee agreed that neither toxicity nor hypercalcemia has been reported for daily intakes <10,000 IU; however, lower values were chosen due to lack of data regarding outcomes with chronic intake. The committee stated that additional research, including better understanding of serum biomarkers, is urgently needed (Aloia, 2011; Institute of Medicine, 2011; Ross *et al.*, 2011).

Many within the medical and research community expressed surprise at these recommendations, since several studies suggest benefit with higher doses. One study estimated a daily need of 3,440 IU for the population to achieve 30 ng/ml serum concentrations. This estimate utilized computer simulation based on data from a six month study of racially diverse adults receiving oral vitamin D supplementation with dosing adjustments performed every two months (Aloia *et al.*, 2008). Another study compared either 1,600 IU daily or 50,000 IU once monthly in community dwelling adults >65 years. None developed toxicity but 19% failed to achieve serum concentrations >30 ng/ml after one year of supplementation (Binkley *et al.*, 2011). Studies regarding fracture prevention have identified an optimal serum concentration of 28-40 ng/ml. Various studies have estimated 700-1000 IU daily for the average elder to achieve this goal; however, much higher doses may be necessary for the entire elder population to achieve these concentrations (Dawson-Hughes, 2004; Dawson-Hughes *et al.*, 2010).

The intramuscular route can also be utilized: ergocalciferol 15 mg IM has been used to treat osteomalacia (Burns and Paterson, 1985).

1.2.7 Serum 25(OH)D measurement

Reported values differ when tested in different laboratories and with different methodologies. Not all labs detect both the 25(OH)D2 and 25(OH)D3 forms. One study sent twenty samples from ten healthy subjects to six laboratories. Each subject contributed one basal sample and one sample spiked with an additional 20 ng/ml 25(OH)D. The labs reported basal sample means of 25(OH)D ranging from 17.1-35.6 ng/ml. The mean increase detected in the samples spiked with 20 ng/ml of additional 25(OH)D ranged from 7.7-18 ng/ml. Individual variation for a single sample between labs ranged from 10-40 ng/ml (Binkley *et al.*, 2004).

Two organizations are working to improve accuracy of testing: The National Institute of Standards and Technology introduced internal standards and the Vitamin D External Quality Assessment Scheme works with analysis problems in individual laboratories (Aloia, 2011).

1.3 Vitamin D: tomorrow

1.3.1 Beyond bone health

The vital role of vitamin D in bone health is universally accepted. However, a plethora of publications over the past several years shows that the role of vitamin D extends far beyond the skeleton. The future of vitamin D will be determined by establishing the non-skeletal benefits of vitamin D and individualizing vitamin D recommendations based on functional outcomes and disease risk. Vitamin D receptors are widely distributed throughout the body, as is the 1α-hydroxylase enzyme responsible for converting 25(OH)D to $1,25(OH)_2D$. Observational data have demonstrated relationships between vitamin D status and infectious, immune, metabolic, degenerative, and neoplastic diseases.

Both strength (see Chapter 6) and falls (see Chapter 4) improve with vitamin D sufficiency. Studies have shown a positive correlation between muscle strength and serum 25(OH)D concentrations in elderly subjects (Bischoff-Ferrari *et al.*, 2004; Holick, 2007; Visser *et al.*, 2003). Other studies have shown less falls with vitamin D intake >600-800 IU/day (Bischoff-Ferrari *et al.*, 2009; Broe *et al.*, 2007; Dawson-Hughes *et al.*, 2010; Holick, 2007; Mason *et al.*, 2011; Murad *et al.*, 2011; Thacher and Clarke, 2011). Although osteomalacia, a painful bony condition, has become rare, non-specific pain is associated with hypovitaminosis D (93% patients with musculoskeletal/bone pain had 25(OH)D <20 ng/ml) (Holick, 2007; Plotnikoff and Quigley, 2003; Thacher and Clarke, 2011).

Observational studies show an inverse relationship between serum concentrations of 25(OH)D and death (Ginde *et al.*, 2009b; Makariou *et al.*, 2011; Thacher and Clarke, 2011; Zittermann *et al.*, 2012). Additionally, subjects taking a vitamin D supplement have decreased mortality compared with controls (Autier and Gandini, 2007; Bjelakovic *et al.*, 2011; Cannell and Hollis, 2008; Makariou *et al.*, 2011; Nadir *et al.*, 2010; Scragg *et al.*, 2007; Thacher and Clarke, 2011).

Vitamin D may have cardiovascular benefits. Observational studies found slightly lower blood pressure in individuals with higher serum 25(OH)D concentrations (Forman *et al.*, 2007; Lind *et al.*, 1995; Makariou *et al.*, 2011; Nadir *et al.*, 2010; Reis *et al.*, 2009; Scragg *et al.*, 2007). Two studies have shown a 9% decrease in systolic blood pressure with initiation of supplemental vitamin D (Islam *et al.*, 2011; Judd *et al.*, 2010; Pfeifer *et al.*, 2001). However, a meta-analysis of 8 studies showed a nonsignificant reduction in SBP and a small but statistically significant decrease in diastolic blood pressure (Witham *et al.*, 2009). The WHI failed to show any blood pressure improvement, but the low compliance of 59% and the low dose of vitamin D may have impacted results (Margolis *et al.*, 2008). Hypertensive individuals exposed to tanning beds became normotensive in 3 months while their serum 25(OH)D concentrations increased >180% (Krause *et al.*, 1998). This relationship may be due to $1,25(OH)_2D$ regulation of the renin-angiotensin system (Li *et al.*, 2002). Although data do not agree, several studies have shown a beneficial relationship between vitamin D adequacy and improved cholesterol values (Carbone *et al.*, 2008; Ford *et al.*, 2005; Hypponen *et al.*, 2008; Lind *et al.*, 1995; Makariou *et al.*, 2011; Martins *et al.*, 2007). NHANES observational data (Kendrick *et al.*, 2009; Kim *et al.*, 2008; Makariou *et al.*, 2011) and a German prospective study (Makariou *et al.*, 2011; Pilz *et al.*, 2008) have both suggested increased risk of cardiovascular disease including stroke or death from congestive heart failure with vitamin D insufficiency. Additional observational studies have demonstrated a relationship between coronary artery disease (Grandi *et al.*, 2010; Kim *et al.*, 2008; Makariou *et al.*, 2011; Temmerman, 2011) or cardiovascular death (Dobnig *et al.*, 2008; Ginde *et al.*, 2009b; Temmerman, 2011; Thacher and Clarke, 2011) and vitamin D insufficiency.

Vitamin D may play a role in both type 1 and type 2 diabetes. A 31-year prospective study of Finish infants given 1000 IU daily for the first year of life (Hypponen *et al.*, 2001) and a meta-analysis of observational studies of children ever receiving vitamin D supplements (Zipitis and Akobeng, 2008) showed a lower risk of development of type 1 diabetes. Additional studies have shown potentially beneficial relationships between vitamin D and type 2 diabetes (Borissova *et*

al., 2003; Chiu *et al.*, 2004; Gannage-Yared *et al.*, 2009; Lind *et al.*, 1995; Nadir *et al.*, 2010; Pittas *et al.*, 2006, 2007a,b; Schwalfenberg, 2008).

The kidney, the site of conversion from 25(OH)D to $1,25(OH)_2D$, is also impacted by vitamin D (see Chapter 11). A meta-analysis showed improved survival in chronic kidney disease patients with higher 25(OH)D serum concentrations (Pilz *et al.*, 2011). NHANES data showed increased prevalence in albuminuria in healthy adults with lower concentrations of serum 25(OH)D (De Boer *et al.*, 2007). Treatment with paricalcitol (a synthetic analog of $1,25(OH)_2D$) decreased proteinuria in patients with chronic kidney disease (Agarwal *et al.*, 2005; Alborzi *et al.*, 2008).

Some have suggested that the seasonality of respiratory infections is related to wintertime hypovitaminosis D (Cannell *et al.*, 2006). An observational study of young Finnish men on a military base found that those with low 25(OH)D serum concentrations (<16 ng/ml) had more absences secondary to respiratory infections (Laaksi *et al.*, 2007). A randomized trial in Japanese schoolchildren showed a reduced incidence of influenza A in those randomized to receive 1200 IU D3 daily compared with those given placebo (Urashima *et al.*, 2010).

Several cross sectional studies reported correlations between serum 25(OH)D concentrations and mood (Bertone-Johnson, 2009). A randomized controlled trial showed significant improvement in mood after one year of vitamin D treatment (Bertone-Johnson, 2009; Jorde *et al.*, 2008). The Finnish birth cohort also showed a decreased incidence in diagnosis of schizophrenia by age 31 in subjects who received vitamin D supplementation during the first year of life (McGrath *et al.*, 2004).

The potential relationships between vitamin D status and neurological conditions including dementia, epilepsy (see Chapter 30), and multiple sclerosis (see Chapter 25) are also of interest. Studies have suggested a decreased risk of multiple sclerosis with vitamin D supplementation (Munger *et al.*, 2004), improved 25(OH)D serum concentrations (Munger *et al.*, 2006; Van Amerongen *et al.*, 2004) or UV exposure. Additionally, a study of 12 patients with multiple sclerosis treated with oral vitamin D supplementation for 28 weeks showed a decrease in gadolinium enhancing lesions on nuclear magnetic brain scan (Kimball *et al.*, 2007). However, another study of 23 individuals randomized to either 6,000 IU or 1000 IU D_2 daily showed no differences in magnetic resonance imaging endpoints after six months (Stein *et al.*, 2011).

The relationship of vitamin D with several different cancers has been investigated. A PubMed search revealed almost 2,000 papers published in the past decade with the medical subject headings terms 'vitamin D' and 'neoplasm'. Research includes cancers of the prostate (see Chapter 23), ovary, lung, pancreas (see Chapter 21), skin, esophagus (see Chapter 24), stomach, endometrium, kidney, and blood with the strongest literature showing a potential benefit for cancers of the breast (Chen *et al.*, 2010; Garland *et al.*, 2007; Gissel *et al.*, 2008; Grant, 2010; World Health Organization International Agency for Research on Cancer, 2008) and colorectum (Fedirko *et al.*, 2010; Gandini *et al.*, 2011; Gorham *et al.*, 2005; 2007; Grant, 2010; Lee *et al.*, 2011;

Ma *et al.*, 2011; Wei *et al.*, 2008; World Health Organization International Agency for Research on Cancer, 2008; Yin *et al.*, 2009; 2011).

Topical $1,25(OH)_2D$ preparations have become firmly established as effective treatment for psoriasis. Additionally, oral $1,25(OH)_2D$ has shown improvement in both skin manifestations and the joint pain of psoriatic arthritis (Holick, 1994; 2007).

The health implications of vitamin D status in pregnant and lactating mothers and their infants are actively being investigated. A case-control study of pregnant women showed a dose-response relationship between serum 25(OH)D and eventual risk of pre-eclampsia during pregnancy (Bodnar *et al.*, 2007).

1.3.2 Personalized medicine

The future of vitamin D will likely bring with it a more individualized approach to assessing vitamin D status and needs. Currently, vitamin D sufficiency is determined by a marker of storage, 25(OH)D. However, there is likely wide individual variation in the functional implications of a given level of 25(OH)D. Furthermore, a fixed amount of vitamin D intake or sun exposure produces a wide individual variation in 25(OH)D response (Aloia *et al.*, 2008; Heaney *et al.*, 2003). Even when all the factors known to influence 25(OH)D concentrations are accounted for, most of the individual variation of 25(OH)D values is difficult to explain. This variation is partially explained by genotypic variants of the vitamin D binding protein, vitamin D receptor, and the hydroxylase enzymes of the vitamin D metabolic pathway (Ahn *et al.*, 2010; Wang *et al.*, 2010). With more widespread use of genotypic analysis, it may be possible to personalize an individual's vitamin D requirement, particularly in view of other disease risk factors. A vitamin D 25-hydroxylase genetic variant has been associated with the risk for type 1 diabetes mellitus (Ramos-Lopez *et al.*, 2007). Genetic variants of the vitamin D receptor may modulate the individual response to vitamin D. Subjects with osteoporosis who showed a response to supplemental calcium and vitamin D expressed a difference in the vitamin D receptor polymorphisms compared with those who did not respond (Elnenaei *et al.*, 2011).

Additional markers of the functional response to vitamin D at the individual level may be more readily available in the future and permit predicting and monitoring the effect of vitamin D supplementation. Measurement of the physiological effects of vitamin D that relate to bone metabolism, the immune system, or other effectors of disease could allow tailoring of the dose of vitamin D to attain maximal physiological benefit. As randomized controlled trials become available confirming the benefits of vitamin D in non-skeletal diseases, the recommended intake of vitamin D and optimal 25(OH)D concentration may need to be adjusted to account for these outcomes. The recommended vitamin D intake may require adjustment for calcium intake, body size, body fat, ethnicity, and usual sun exposure. As individualized medicine becomes more of a reality in all areas of medical care, the optimal role of vitamin D will certainly be better defined.

1.3.3 So much more to learn!

Although our knowledge of vitamin D has grown exponentially since ancient descriptions of rickets, many questions remain. Individuals and organizations have posed several questions still needing to be answered.

In October 2011, scientists, government agents, and academics, including representatives from the CDC and American Academy of Pediatrics, gathered for 'A Vitamin D Expert Panel Meeting' to examine current knowledge and future research needs. They stressed that public health recommendations should consider health implications beyond rickets and that more longitudinal studies are needed (Scanlon, 2001).

Similarly, National Institute of Health held a conference entitled 'Vitamin D and health in the 21st century: bone and beyond' in 2003. Along with the National Institute of Health members, participants included representatives from national institutes (National Cancer Institute, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Diabetes and Digestive and Kidney Disorders), government departments (Department of Agriculture, Office of Dietary Supplements, CDC) and industry (National Dairy Council, Coca-Cola). They identified several important areas in need of research including developing universal definitions of adequacy based on health outcomes, determination of dietary/supplement/UV requirements including better understanding of safety and toxicity in all ages and ethnic groups (Raiten and Picciano, 2004).

Vitamin D has exciting potential, and a flurry of research activity is now underway to explore its potential benefits. Enthusiasm regarding vitamin D is reminiscent of the health benefits attributed to other vitamins in the past, many of which were not eventually confirmed. Vitamin D differs from other vitamins in that it is a prohormone and not a true vitamin. The future is bound to bring clarification of the optimal role of vitamin D and new applications for vitamin D in health and disease.

References

Agarwal, R., Acharya, M., Tian, J., Hippensteel, R.L., Melnick, J.Z., Qiu, P., Williams, L. and Batlle, D., 2005. Antiproteinuric effect of oral paricalcitol in chronic kidney disease. Kidney International 68, 2823-2828.

Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K.C., McCullough, M.L., Gallicchio, L., Jacobs, E.J., Ascherio, A., Helzlsouer, K., Jacobs, K.B., Li, Q., Weinstein, S.J., Purdue, M., Virtamo, J., Horst, R., Wheeler, W., Chanock, S., Hunter, D.J., Hayes, R.B., Kraft, P. and Albanes, D., 2010. Genome-wide association study of circulating vitamin D levels. Human Molecular Genetics 19, 2739-2745.

Alborzi, P., Patel, N.A., Peterson, C., Bills, J.E., Bekele, D.M., Bunaye, Z., Light, R.P. and Agarwal, R., 2008. Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. Hypertension 52, 249-255.

Aloia, J.F., 2011. Clinical review: the 2011 report on dietary reference intake for vitamin D: where do we go from here? The Journal of Clinical Endocrinology and Metabolism 96, 2987-2996.

Aloia, J.F., Patel, M., Dimaano, R., Li-Ng, M., Talwar, S.A., Mikhail, M., Pollack, S. and Yeh, J.K., 2008. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. American Journal of Clinical Nutrition 87, 1952-1958.

Autier, P. and Gandini, S., 2007. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Archives of Internal Medicine 167, 1730-1737.

Barger-Lux, M.J., Heaney, R.P., Dowell, S., Chen, T.C. and Holick, M.F., 1998. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporosis International 8, 222-230.

Bell, D.S., 2011. Protean manifestations of vitamin D deficiency, part 1: the epidemic of deficiency. Southern MedicalJournal 104, 331-334.

Bertone-Johnson, E.R., 2009. Vitamin D and the occurrence of depression: causal association or circumstantial evidence? Nutrition Reviews 67, 481-492.

Binkley, N., Gemar, D., Engelke, J., Gangnon, R., Ramamurthy, R., Krueger, D. and Drezner, M.K., 2011. Evaluation of ergocalciferol or cholecalciferol dosing, 1,600 IU daily or 50,000 IU monthly in older adults. Journal of Clinical Endocrinology and Metabolism 96, 981-988.

Binkley, N., Krueger, D., Cowgill, C.S., Plum, L., Lake, E., Hansen, K.E., DeLuca, H.F. and Drezner, M.K., 2004. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. Journal of Clinical Endocrinology and Metabolism 89, 3152-3157.

Binkley, N., Novotny, R., Krueger, D., Kawahara, T., Daida, Y.G., Lensmeyer, G., Hollis, B.W. and Drezner, M.K., 2007. Low vitamin D status despite abundant sun exposure. The Journal of Clinical Endocrinology and Metabolism 92, 2130-2135.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Staehelin, H.B., Orav, J.E., Stuck, A.E., Theiler, R., Wong, J.B., Egli, A., Kiel, D.P. and Henschkowski, J., 2009. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ 339, b3692.

Bischoff-Ferrari, H.A., Dietrich, T., Orav, E.J., Hu, F.B., Zhang, Y., Karlson, E.W. and Dawson-Hughes, B., 2004. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged ≥60 y. The American Journal of Clinical Nutrition 80, 752-758.

Bjelakovic, G., Gluud, L.L., Nikolova, D., Whitfield, K., Wetterslev, J., Simonetti, R.G., Bjelakovic, M. and Gluud, C., 2011. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database of Systematic Reviews, CD007470.

Blunt, J.W., DeLuca, H.F. and Schnoes, H.K., 1968. 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D3. Biochemistry 7, 3317-3322.

Bodnar, L.M., Catov, J.M., Simhan, H.N., Holick, M.F., Powers, R.W. and Roberts, J.M., 2007. Maternal vitamin D deficiency increases the risk of preeclampsia. Journal of Clinical Endocrinology and Metabolism 92, 3517-3522.

Borissova, A.M., Tankova, T., Kirilov, G., Dakovska, L. and Kovacheva, R., 2003. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. International Journal of Clinical Practice 57, 258-261.

Broe, K.E., Chen, T.C., Weinberg, J., Bischoff-Ferrari, H.A., Holick, M.F. and Kiel, D.P., 2007. A higher dose of vitamin d reduces the risk of falls in nursing home residents: a randomized, multiple-dose study. Journal of the American Geriatrics Society 55, 234-239.

Burns, J. and Paterson, C.R., 1985. Single dose vitamin D treatment for osteomalacia in the elderly. British Medical Journal 290, 281-282.

Cannell, J.J. and Hollis, B.W., 2008. Use of vitamin D in clinical practice. Alternative Medicine Review 13, 6-20.

Cannell, J.J., Vieth, R., Umhau, J.C., Holick, M.F., Grant, W.B., Madronich, S., Garland, C.F. and Giovannucci, E., 2006. Epidemic influenza and vitamin D. Epidemiology and Infection 134, 1129-1140.

Carbone, L.D., Rosenberg, E.W., Tolley, E.A., Holick, M.F., Hughes, T.A., Watsky, M.A., Barrow, K.D., Chen, T.C., Wilkin, N.K., Bhattacharya, S.K., Dowdy, J.C., Sayre, R.M. and Weber, K.T., 2008. 25-hydroxyvitamin D, cholesterol, and ultraviolet irradiation. Metabolism: Clinical and Experimental 57, 741-748.

Centers for Disease Control and Prevention, 2001. Severe malnutrition among young children – Georgia, January 1997-June 1999. Morbidity and Mortality Weekly Report 50, 224-227.

Cheadle, W., 1988. A discussion on rickets. BMJ II, 1145-1148.

Chen, P., Hu, P., Xie, D., Qin, Y., Wang, F. and Wang, H., 2010. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. Breast Cancer Research and Treatment 121, 469-477.

Chesney, R.W., 2012. Theobald palm and his remarkable observation: how the sunshine vitamin came to be recognized. Nutrients 4, 42-51.

Chick, H., Dalyell, E., Hume, M., Mackay, H.M.M., Henderson Smith, H., 1922. The aetiology of rickets in infants: prophylactic and curative observations at the Vienna University Kinderklinik. Lancet ii, 7-11.

Chiu, K.C., Chu, A., Go, V.L. and Saad, M.F., 2004. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. The American Journal of Clinical Nutrition 79, 820-825.

Cosgrove, L. and Dietrich, A., 1985. Nutritional rickets in breast-fed infants. The Journal of Family Practice 21, 205-209.

Dawson-Hughes, B., 2004. Racial/ethnic considerations in making recommendations for vitamin D for adult and elderly men and women. The American Journal of Clinical Nutrition 80, 1763S-1766S.

Dawson-Hughes, B., Mithal, A., Bonjour, J.P., Boonen, S., Burckhardt, P., Fuleihan, G.E., Josse, R.G., Lips, P., Morales-Torres, J. and Yoshimura, N., 2010. IOF position statement: vitamin D recommendations for older adults. Osteoporosis International 21, 1151-1154.

De Boer, I.H., Ioannou, G.N., Kestenbaum, B., Brunzell, J.D. and Weiss, N.S., 2007. 25-hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Kidney Diseases 50, 69-77.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., Kinkeldei, J., Boehm, B.O., Weihrauch, G. and Maerz, W., 2008. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Archives of Internal Medicine 168, 1340-1349.

El-Hajj Fuleihan, G., Nabulsi, M., Choucair, M., Salamoun, M., Hajj Shahine, C., Kizirian, A. and Tannous, R., 2001. Hypovitaminosis D in healthy schoolchildren. Pediatrics 107, E53.

Elnenaei, M.O., Chandra, R., Mangion, T. and Moniz, C., 2011. Genomic and metabolomic patterns segregate with responses to calcium and vitamin D supplementation. British Journal of Nutrition 105, 71-79.

Fedirko, V., Bostick, R.M., Goodman, M., Flanders, W.D. and Gross, M.D., 2010. Blood 25-hydroxyvitamin D3 concentrations and incident sporadic colorectal adenoma risk: a pooled case-control study. American Journal of Epidemiology 172, 489-500.

Findlay, L., 1908. The etiology of rickets: a clinical and experimental study. British Medical Journal 2, 13-17.

Foote, J.A., 1927. Evidence of rickets prior to 1650. American Journal of Diseases of Children 34, 45-61.

Ford, E.S., Ajani, U.A., McGuire, L.C. and Liu, S., 2005. Concentrations of serum vitamin D and the metabolic syndrome among US adults. Diabetes Care 28, 1228-1230.

Forman, J.P., Giovannucci, E., Holmes, M.D., Bischoff-Ferrari, H.A., Tworoger, S.S., Willett, W.C. and Curhan, G.C., 2007. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 49, 1063-1069.

Forrest, K.Y. and Stuhldreher, W.L., 2011. Prevalence and correlates of vitamin D deficiency in US adults. Nutrition Research 31, 48-54.

Fraser, D.R. and Kodicek, E., 1970. Unique biosynthesis by kidney of a biological active vitamin D metabolite. Nature 228, 764-766.

Gandini, S., Boniol, M., Haukka, J., Byrnes, G., Cox, B., Sneyd, M.J., Mullie, P. and Autier, P., 2011. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. International journal of cancer. Journal International du Cancer 128, 1414-1424.

Gannage-Yared, M.H., Chedid, R., Khalife, S., Azzi, E., Zoghbi, F. and Halaby, G., 2009. Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. European Journal of Endocrinology 160, 965-971.

Garland, C.F., Gorham, E.D., Mohr, S.B., Grant, W.B., Giovannucci, E.L., Lipkin, M., Newmark, H., Holick, M.F. and Garland, F.C., 2007. Vitamin D and prevention of breast cancer: pooled analysis. Journal of Steroid Biochemistry and Molecular Biology 103, 708-711.

Ginde, A.A., Liu, M.C. and Camargo Jr., C.A., 2009a. Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. Archives of Internal Medicine 169, 626-632.

Ginde, A.A., Scragg, R., Schwartz, R.S. and Camargo Jr., C.A., 2009b. Prospective study of serum 25-hydroxyvitamin D level, cardiovascular disease mortality, and all-cause mortality in older US adults. Journal of the American Geriatrics Society 57, 1595-1603.

Gissel, T., Rejnmark, L., Mosekilde, L. and Vestergaard, P., 2008. Intake of vitamin D and risk of breast cancer – a meta-analysis. Journal of Steroid Biochemistry and Molecular Biology 111, 195-199.

Gordon, C.M., DePeter, K.C., Feldman, H.A., Grace, E. and Emans, S.J., 2004. Prevalence of vitamin D deficiency among healthy adolescents. Archives of Pediatrics and Adolescent Medicine 158, 531-537.

Gordon, C.M., Feldman, H.A., Sinclair, L., Williams, A.L., Kleinman, P.K., Perez-Rossello, J. and Cox, J.E., 2008. Prevalence of vitamin D deficiency among healthy infants and toddlers. Archives of Pediatrics and Adolescent Medicine 162, 505-512.

Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M., Newmark, H.L., Giovannucci, E., Wei, M. and Holick, M.F., 2005. Vitamin D and prevention of colorectal cancer. The Journal of Steroid Biochemistry and Molecular Biology 97, 179-194.

Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M., Newmark, H.L., Giovannucci, E., Wei, M. and Holick, M.F., 2007. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. American Journal of Preventive Medicine 32, 210-216.

Grandi, N.C., Breitling, L.P. and Brenner, H., 2010. Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. Preventive Medicine 51, 228-233.

Grant, W.B., 2010. Relation between prediagnostic serum 25-hydroxyvitamin D level and incidence of breast, colorectal, and other cancers. Journal of photochemistry and photobiology. B, Biology 101, 130-136.

Guy, R., 1923. The history of cod liver oil as a remedy. American Journal of Diseases of Children 26, 112-116.

Haussler, M.R., Myrtle, J.F. and Norman, A.W., 1968. The association of a metabolite of vitamin D3 with intestinal mucosa chromatin *in vivo*. Journal of Biological Chemistry 243, 4055-4064.

Heaney, R.P., 2004. Functional indices of vitamin D status and ramifications of vitamin D deficiency. American Journal of Clinical Nutrition 80, 1706S-1709S.

Heaney, R.P., Davies, K.M., Chen, T.C., Holick, M.F. and Barger-Lux, M.J., 2003. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. American Journal of Clinical Nutrition 77, 204-210.

Heaney, R.P. and Holick, M.F., 2011. Why the IOM recommendations for vitamin D are deficient. Journal of Bone and Mineral Research 26, 455-457.

Hess, A.F., 1924. On the Induction of Antirachitic Properties in Rations by Exposure to Light. Science 60, 269.

Hess, A.F.W., Mildred, 1925a. The antirachitic value of irradiated cholesterol and phytosterol. Journal of Biological Chemistry 64, 181-191.

Hess, A.F.W., Mildred, 1925b. A study of light waves in their relation to rickets. JAMA 80, 687-690.

Highwood, E.J. and Kinnersley, R.P., 2006. When smoke gets in our eyes: the multiple impacts of atmospheric black carbon on climate, air quality and health. Environment International 32, 560-566.

Holick, M.F., 1994. McCollum Award Lecture, 1994: vitamin D – new horizons for the 21st century. American Journal of Clinical Nutrition 60, 619-630.

Holick, M.F., 2003. Vitamin D: A millenium perspective. Journal of Cellular Biochemistry 88, 296-307.

Holick, M.F., 2006. High prevalence of vitamin D inadequacy and implications for health. Mayo Clinic Proceedings 81, 353-373.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Holick, M.F. and Chen, T.C., 2008. Vitamin D deficiency: a worldwide problem with health consequences. American Journal of Clinical Nutrition 87, 1080S-1086S.

Holick, M.F., MacLaughlin, J.A., Clark, M.B., Holick, S.A., Potts Jr., J.T., Anderson, R.R., Blank, I.H., Parrish, J.A. and Elias, P., 1980. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science 210, 203-205.

Holick, M.F., Matsuoka, L.Y. and Wortsman, J., 1989. Age, vitamin D, and solar ultraviolet. Lancet 2, 1104-1105.

Holick, M.F., Schnoes, H.K., DeLuca, H.F., Suda, T. and Cousins, R.J., 1971. Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. Biochemistry 10, 2799-2804.

Holick, M.F., Shao, Q., Liu, W.W. and Chen, T.C., 1992. The vitamin D content of fortified milk and infant formula. New England Journal of Medicine 326, 1178-1181.

Hypponen, E., Boucher, B.J., Berry, D.J. and Power, C., 2008. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. Diabetes 57, 298-305.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R. and Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358, 1500-1503.

Hypponen, E. and Power, C., 2007. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. American Journal of Clinical Nutrition 85, 860-868.

Institute of Medicine, 2011. Dietary reference intakes for calcium and vitamin D. The National Academies Press, Washington, DC, USA.

Isaia, G., Giorgino, R., Rini, G.B., Bevilacqua, M., Maugeri, D. and Adami, S., 2003. Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. Osteoporosis International 14, 577-582.

Islam, T., Peiris, P., Copeland, R.J., El Zoghby, M. and Peiris, A.N., 2011. Vitamin D: Lessons from the veterans population. Journal of the American Medical Directors Association 12, 257-262.

Jorde, R., Sneve, M., Figenschau, Y., Svartberg, J. and Waterloo, K., 2008. Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. Journal of Internal Medicine 264, 599-609.

Judd, S.E., Raiser, S.N., Kumari, M. and Tangpricha, V., 2010. 1,25-dihydroxyvitamin D3 reduces systolic blood pressure in hypertensive adults: a pilot feasibility study. The Journal of Steroid Biochemistry and Molecular Biology 121, 445-447.

Kendrick, J., Targher, G., Smits, G. and Chonchol, M., 2009. 25-hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis 205, 255-260.

Kim, D.H., Sabour, S., Sagar, U.N., Adams, S. and Whellan, D.J., 2008. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). American Journal of Cardiology 102, 1540-1544.

Kimball, S.M., Ursell, M.R., O'Connor, P. and Vieth, R., 2007. Safety of vitamin D3 in adults with multiple sclerosis. American Journal of Clinical Nutrition 86, 645-651.

Krause, R., Buhring, M., Hopfenmuller, W., Holick, M.F. and Sharma, A.M., 1998. Ultraviolet B and blood pressure. Lancet 352, 709-710.

Kumar, J., Muntner, P., Kaskel, F.J., Hailpern, S.M. and Melamed, M.L., 2009. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. Pediatrics 124, e362-370.

Laaksi, I., Ruohola, J.P., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamaki, H. and Ylikomi, T., 2007. An association of serum vitamin D concentrations <40 nmol/l with acute respiratory tract infection in young Finnish men. American Journal of Clinical Nutrition 86, 714-717.

Lawson, D.E., Fraser, D.R., Kodicek, E., Morris, H.R. and Williams, D.H., 1971. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. Nature 230, 228-230.

Lawson, D.E., Wilson, P.W. and Kodicek, E., 1969. New vitamin D metabolite localized in intestinal cell nuclei. Nature 222, 171-172.

Lee, J.E., Li, H., Chan, A.T., Hollis, B.W., Lee, I.M., Stampfer, M.J., Wu, K., Giovannucci, E. and Ma, J., 2011. Circulating levels of vitamin D and colon and rectal cancer: the Physicians' Health Study and a meta-analysis of prospective studies. Cancer Prevention Research 4, 735-743.

Lee, J.H., O'Keefe, J.H., Bell, D., Hensrud, D.D. and Holick, M.F., 2008. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? Journal of the American College of Cardiology 52, 1949-1956.

Lee, J.M., Smith, J.R., Philipp, B.L., Chen, T.C., Mathieu, J. and Holick, M.F., 2007. Vitamin D deficiency in a healthy group of mothers and newborn infants. Clinical Pediatrics 46, 42-44.

Li, Y.C., Kong, J., Wei, M., Chen, Z.F., Liu, S.Q. and Cao, L.P., 2002. 1,25-dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. Journal of Clinical Investigation 110, 229-238.

Lind, L., Hanni, A., Lithell, H., Hvarfner, A., Sorensen, O.H. and Ljunghall, S., 1995. Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. American Journal of Hypertension 8, 894-901.

Lowe, N.M., Mitra, S.R., Foster, P.C., Bhojani, I. and McCann, J.F., 2010. Vitamin D status and markers of bone turnover in Caucasian and South Asian postmenopausal women living in the UK. British Journal of Nutrition 103, 1706-1710.

Ma, Y., Zhang, P., Wang, F., Yang, J., Liu, Z. and Qin, H., 2011. Association between vitamin D and risk of colorectal cancer: a systematic review of prospective studies. Journal of Clinical Oncology 29, 3775-3782.

Macintyre, I., Evans, I.M. and Larkins, R.G., 1977. Vitamin D. Clinical Endocrinology 6, 65-79.

MacLaughlin, J. and Holick, M.F., 1985. Aging decreases the capacity of human skin to produce vitamin D3. Journal of Clinical Investigation 76, 1536-1538.

Makariou, S., Liberopoulos, E.N., Elisaf, M. and Challa, A., 2011. Novel roles of vitamin D in disease: what is new in 2011? European Journal of Internal Medicine 22, 355-362.

Margolis, K.L., Ray, R.M., Van Horn, L., Manson, J.E., Allison, M.A., Black, H.R., Beresford, S.A., Connelly, S.A., Curb, J.D., Grimm Jr., R.H., Kotchen, T.A., Kuller, L.H., Wassertheil-Smoller, S., Thomson, C.A. and Torner, J.C., 2008. Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. Hypertension 52, 847-855.

Martins, D., Wolf, M., Pan, D., Zadshir, A., Tareen, N., Thadhani, R., Felsenfeld, A., Levine, B., Mehrotra, R. and Norris, K., 2007. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Archives of Internal Medicine 167, 1159-1165.

Marwaha, R.K., Tandon, N., Reddy, D.R., Aggarwal, R., Singh, R., Sawhney, R.C., Saluja, B., Ganie, M.A. and Singh, S., 2005. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. American Journal of Clinical Nutrition 82, 477-482.

Mason, R.S., Sequeira, V.B. and Gordon-Thomson, C., 2011. Vitamin D: the light side of sunshine. European Journal of Clinical Nutrition 65, 986-993.

Matsuoka, L.Y., Ide, L., Wortsman, J., MacLaughlin, J.A. and Holick, M.F., 1987. Sunscreens suppress cutaneous vitamin D3 synthesis. The Journal of Clinical Endocrinology and Metabolism 64, 1165-1168.

Matsuoka, L.Y., Wortsman, J., Hanifan, N. and Holick, M.F., 1988. Chronic sunscreen use decreases circulating concentrations of 25-hydroxyvitamin D. A preliminary study. Archives of Dermatology 124, 1802-1804.

Mayer, J., 1957. Armand Trousseau and the arrow of time. Nutrition Reviews 15, 321-323.

McCollum, E.V., Simmonds, N., Becker, E. and Shipley, P.G., 1922. An experimental demonstration of a vitamin which promotes calcium deposition. Journal of Biological Chemistry, 293-312.

McCollum, E.V., Simmonds, N., Becker, Shipley, P.G. and Park, E.A., 1921. The production of rickets by diets low in phosphorus and fat-solube vitamin A. Journal of Biological Chemistry, 507-528.

McGrath, J., Saari, K., Hakko, H., Jokelainen, J., Jones, P., Jarvelin, M.R., Chant, D. and Isohanni, M., 2004. Vitamin D supplementation during the first year of life and risk of schizophrenia: a Finnish birth cohort study. Schizophrenia Research 67, 237-245.

Mellanby, E., 1919. An experimental investigation on rickets. Lancet 1, 407-412.

Mims, F.M., 3rd, 1996. Significant reduction of UVB caused by smoke from biomass burning in Brazil. Photochemistry and Photobiology 64, 814-816.

Munger, K.L., Levin, L.I., Hollis, B.W., Howard, N.S. and Ascherio, A., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296, 2832-2838.

Munger, K.L., Zhang, S.M., O'Reilly, E., Hernan, M.A., Olek, M.J., Willett, W.C. and Ascherio, A., 2004. Vitamin D intake and incidence of multiple sclerosis. Neurology 62, 60-65.

Murad, M.H., Elamin, K.B., Abu Elnour, N.O., Elamin, M.B., Alkatib, A.A., Fatourechi, M.M., Almandoz, J.P., Mullan, R.J., Lane, M.A., Liu, H., Erwin, P.J., Hensrud, D.D. and Montori, V.M., 2011. Clinical review: The effect of vitamin D on falls: a systematic review and meta-analysis. Journal of Clinical Endocrinology and Metabolism 96, 2997-3006.

Murphy, S.C., Whited, L.J., Rosenberry, L.C., Hammond, B.H., Bandler, D.K. and Boor, K.J., 2001. Fluid milk vitamin fortification compliance in New York State. Journal of Dairy Science 84, 2813-2820.

Nadir, M.A., Szwejkowski, B.R. and Witham, M.D., 2010. Vitamin D and cardiovascular prevention. Cardiovascular Therapeutics 28, e5-12.

Need, A.G., O'Loughlin, P.D., Morris, H.A., Coates, P.S., Horowitz, M. and Nordin, B.E., 2008. Vitamin D metabolites and calcium absorption in severe vitamin D deficiency. Journal of Bone and Mineral Research 23, 1859-1863.

Nield, L.S., Mahajan, P., Joshi, A. and Kamat, D., 2006. Rickets: not a disease of the past. American Family Physician 74, 619-626.

Norman, A.W., Lund, J. and Deluca, H.F., 1964. Biologically active forms of vitamin D3 in kidney and intestine. Archives of Biochemistry and Biophysics 108, 12-21.

Norman, A.W., Myrtle, J.F., Midgett, R.J., Nowicki, H.G., Williams, V. and Popjak, G., 1971. 1,25-dihydroxycholecalciferol: identification of the proposed active form of vitamin D3 in the intestine. Science 173, 51-54.

O'Riordan, J.L., 1997. Rickets, from history to molecular biology, from monkeys to YACS. Journal of Endocrinology 154 Suppl, S3-13.

O'Riordan, J.L., 2006. Rickets in the 17th century. Journal of Bone and Mineral Research 21, 1506-1510.

Orwoll, E., Nielson, C.M., Marshall, L.M., Lambert, L., Holton, K.F., Hoffman, A.R., Barrett-Connor, E., Shikany, J.M., Dam, T. and Cauley, J.A., 2009. Vitamin D deficiency in older men. Journal of Clinical Endocrinology and Metabolism 94, 1214-1222.

Park, E.A., 1923. The etiology of rickets. Physiological Reviews 3, 106-163.

Passeri, G., Pini, G., Troiano, L., Vescovini, R., Sansoni, P., Passeri, M., Gueresi, P., Delsignore, R., Pedrazzoni, M. and Franceschi, C., 2003. Low vitamin D status, high bone turnover, and bone fractures in centenarians. Journal of Clinical Endocrinology and Metabolism 88, 5109-5115.

Pepper, K.J., Judd, S.E., Nanes, M.S. and Tangpricha, V., 2009. Evaluation of vitamin D repletion regimens to correct vitamin D status in adults. Endocrine Practice 15, 95-103.

Pfeifer, M., Begerow, B., Minne, H.W., Nachtigall, D. and Hansen, C., 2001. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. Journal of Clinical Endocrinology and Metabolism 86, 1633-1637.

Pilz, S., Dobnig, H., Fischer, J.E., Wellnitz, B., Seelhorst, U., Boehm, B.O. and Marz, W., 2008. Low vitamin d levels predict stroke in patients referred to coronary angiography. Stroke 39, 2611-2613.

Pilz, S., Iodice, S., Zittermann, A., Grant, W.B. and Gandini, S., 2011. Vitamin D status and mortality risk in CKD: a meta-analysis of prospective studies. American Journal of Kidney Diseases 58, 374-382.

Pittas, A.G., Dawson-Hughes, B., Li, T., Van Dam, R.M., Willett, W.C., Manson, J.E. and Hu, F.B., 2006. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 29, 650-656.

Pittas, A.G., Harris, S.S., Stark, P.C. and Dawson-Hughes, B., 2007a. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 30, 980-986.

Pittas, A.G., Lau, J., Hu, F.B. and Dawson-Hughes, B., 2007b. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. Journal of Clinical Endocrinology and Metabolism 92, 2017-2029.

Plotnikoff, G.A. and Quigley, J.M., 2003. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clinic Proceedings 78, 1463-1470.

Ponchon, G. and DeLuca, H.F., 1969. The role of the liver in the metabolism of vitamin D. Journal of Clinical Investigation 48, 1273-1279.

Raiten, D.J. and Picciano, M.F., 2004. Vitamin D and health in the 21st century: bone and beyond. Executive summary. American Journal of Clinical Nutrition 80, 1673S-1677S.

Rajakumar, K., 2003. Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. Pediatrics 112, e132-135.

Ramos-Lopez, E., Bruck, P., Jansen, T., Herwig, J. and Badenhoop, K., 2007. CYP2R1 (vitamin D 25-hydroxylase) gene is associated with susceptibility to type 1 diabetes and vitamin D levels in Germans. Diabetes/Metabolism Research and Reviews 23, 631-636.

Reis, J.P., von Muhlen, D., Miller, E.R., 3rd, Michos, E.D. and Appel, L.J., 2009. Vitamin D status and cardiometabolic risk factors in the United States adolescent population. Pediatrics 124, e371-379.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. Journal of Clinical Endocrinology and Metabolism 96, 53-58.

Rovner, A.J. and O'Brien, K.O., 2008. Hypovitaminosis D among healthy children in the United States: a review of the current evidence. Archives of Pediatrics and Adolescent Medicine 162, 513-519.

Scanlon, K.S., 2001. Vitamin D Expert Panel Meeting, Atlanta. Available at: http://www.cdc.gov/nccdphp/dnpa/nutrition/pdf/Vitamin_D_Expert_Panel_Meeting.pdf.

Schneider, H.A., 1973. Harry Steenbock (1886-1967) – a biographical sketch. Journal of Nutrition 103, 1233-1247.

Schwalfenberg, G., 2008. Vitamin D and diabetes: improvement of glycemic control with vitamin D3 repletion. Canadian Family Physician 54, 864-866.

Scragg, R., Sowers, M. and Bell, C., 2007. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. American Journal of Hypertension 20, 713-719.

Sedrani, S.H., 1984. Low 25-hydroxyvitamin D and normal serum calcium concentrations in Saudi Arabia: Riyadh region. Annals of Nutrition and Metabolism 28, 181-185.

Seth, A., Marwaha, R.K., Singla, B., Aneja, S., Mehrotra, P., Sastry, A., Khurana, M.L., Mani, K., Sharma, B. and Tandon, N., 2009. Vitamin D nutritional status of exclusively breast fed infants and their mothers. Journal of Pediatric Endocrinology and Metabolism 22, 241-246.

Smerdon, G.T., 1950. Daniel Whistler and the English disease: a translation and biographical note. Journal of the History of Medicine, 397-415.

Steenbock, H., 1924. The induction of growth promoting and calcifying properties in a ration by exposure to light. Science 60, 224-225.

Steenbock, H. and Black, A., 1924. The induction of growth promoting and calcifying properties in a ration by exposure to ultraviolet light. Journal of Biological Chemistry 61, 405-422.

Steenbock, H. and Nelson, M.T., 1924. The induction of calcifying properties in rickets-producing ration by radiant energy. Journal of Biological Chemistry 62, 209-216.

Stein, M.S., Liu, Y., Gray, O.M., Baker, J.E., Kolbe, S.C., Ditchfield, M.R., Egan, G.F., Mitchell, P.J., Harrison, L.C., Butzkueven, H. and Kilpatrick, T.J., 2011. A randomized trial of high-dose vitamin D2 in relapsing-remitting multiple sclerosis. Neurology 77, 1611-1618.

Stumpf, W.E., Sar, M., Reid, F.A., Tanaka, Y. and DeLuca, H.F., 1979. Target cells for 1,25-dihydroxyvitamin D3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. Science 206, 1188-1190.

Sullivan, S.S., Rosen, C.J., Halteman, W.A., Chen, T.C. and Holick, M.F., 2005. Adolescent girls in Maine are at risk for vitamin D insufficiency. Journal of the American Dietetic Association 105, 971-974.

Taha, S.A., Dost, S.M. and Sedrani, S.H., 1984. 25-hydroxyvitamin D and total calcium: extraordinarily low plasma concentrations in Saudi mothers and their neonates. Pediatric Research 18, 739-741.

Temmerman, J.C., 2011. Vitamin D and cardiovascular disease. Journal of the American College of Nutrition 30, 167-170.

Thacher, T.D. and Abrams, S.A., 2010. Relationship of calcium absorption with 25(OH)D and calcium intake in children with rickets. Nutrition Reviews 68, 682-688.

Thacher, T.D. and Clarke, B.L., 2011. Vitamin D insufficiency. Mayo Clinic Proceedings 86, 50-60.

Thacher, T.D., Fischer, P.R., Strand, M.A. and Pettifor, J.M., 2006. Nutritional rickets around the world: causes and future directions. Annals of Tropical Paediatrics 26, 1-16.

Thomas, M.K., Lloyd-Jones, D.M., Thadhani, R.I., Shaw, A.C., Deraska, D.J., Kitch, B.T., Vamvakas, E.C., Dick, I.M., Prince, R.L. and Finkelstein, J.S., 1998. Hypovitaminosis D in medical inpatients. New England Journal of Medicine 338, 777-783.

Tsiaras, W.G. and Weinstock, M.A., 2011. Factors influencing vitamin D status. Acta Dermato-Venereologica 91, 115-124.

US Department of Health and Human Services, 2000. HealthyPeople 2010. USDHHS, Washington, DC, USA. Available at: http://www.cdc.gov/nchs/healthy_people.htm.

Urashima, M., Segawa, T., Okazaki, M., Kurihara, M., Wada, Y. and Ida, H., 2010. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. American Journal of Clinical Nutrition 91, 1255-1260.

Van Schoor, N.M. and Lips, P., 2011. Worldwide vitamin D status. Best practice & research. Clinical Endocrinology and Metabolism 25, 671-680.

VanAmerongen, B.M., Dijkstra, C.D., Lips, P. and Polman, C.H., 2004. Multiple sclerosis and vitamin D: an update. European Journal of Clinical Nutrition 58, 1095-1109.

Visser, M., Deeg, D.J. and Lips, P., 2003. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. Journal of Clinical Endocrinology and Metabolism 88, 5766-5772.

Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., van Meurs, J.B., Berry, D., Kiel, D.P., Streeten, E.A., Ohlsson, C., Koller, D.L., Peltonen, L., Cooper, J.D., O'Reilly, P.F., Houston, D.K., Glazer, N.L., Vandenput, L., Peacock, M., Shi, J., Rivadeneira, F., McCarthy, M.I., Anneli, P., de Boer, I.H., Mangino, M., Kato, B., Smyth, D.J., Booth, S.L., Jacques, P.F., Burke, G.L., Goodarzi, M., Cheung, C.L., Wolf, M., Rice, K., Goltzman, D., Hidiroglou, N., Ladouceur, M., Wareham, N.J., Hocking, L.J., Hart, D., Arden, N.K., Cooper, C., Malik, S., Fraser, W.D., Hartikainen, A.L., Zhai, G., Macdonald, H.M., Forouhi, N.G., Loos, R.J., Reid, D.M., Hakim, A., Dennison, E., Liu, Y., Power, C., Stevens, H.E., Jaana, L., Vasan, R.S., Soranzo, N., Bojunga, J., Psaty, B.M., Lorentzon, M., Foroud, T., Harris, T.B., Hofman, A., Jansson, J.O., Cauley, J.A., Uitterlinden, A.G., Gibson, Q., Jarvelin, M.R., Karasik, D., Siscovick, D.S., Econs, M.J., Kritchevsky, S.B., Florez, J.C., Todd, J.A., Dupuis, J., Hypponen, E. and Spector, T.D., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180-188.

Ward, L.M., Gaboury, I., Ladhani, M. and Zlotkin, S., 2007. Vitamin D-deficiency rickets among children in Canada. CMAJ 177, 161-166.

Webb, A.R., Kline, L. and Holick, M.F., 1988. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. Journal of Clinical Endocrinology and Metabolism 67, 373-378.

Wei, M.Y., Garland, C.F., Gorham, E.D., Mohr, S.B. and Giovannucci, E., 2008. Vitamin D and prevention of colorectal adenoma: a meta-analysis. Cancer Epidemiology, Biomarkers and Prevention 17, 2958-2969.

Weisberg, P., Scanlon, K.S., Li, R. and Cogswell, M.E., 2004. Nutritional rickets among children in the United States: review of cases reported between 1986 and 2003. American Journal of Clinical Nutrition 80, 1697S-1705S.

Wisconsin Alumni Research Foundation, Steenbock and WARF's Founding. Available at: http://www.warf.org/about/index.jsp?cid=26&scid=33.

Witham, M.D., Nadir, M.A. and Struthers, A.D., 2009. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. Journal of Hypertension 27, 1948-1954.

World Health Organization International Agency for Research on Cancer, 2008. Vitamin D and Cancer. Available at: http://www.iarc.fr/en/publications/pdfs-online/wrk/wrk5/Report_VitD.pdf.

Wortsman, J., Matsuoka, L.Y., Chen, T.C., Lu, Z. and Holick, M.F., 2000. Decreased bioavailability of vitamin D in obesity. American Journal of Clinical Nutrition 72, 690-693.

Yetley, E.A., 2008. Assessing the vitamin D status of the US population. American Journal of Clinical Nutrition 88, 558S-564S.

Yin, L., Grandi, N., Raum, E., Haug, U., Arndt, V. and Brenner, H., 2009. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. Alimentary Pharmacology and Therapeutics 30, 113-125.

Yin, L., Grandi, N., Raum, E., Haug, U., Arndt, V. and Brenner, H., 2011. Meta-analysis: serum vitamin D and colorectal adenoma risk. Preventive Medicine 53, 10-16.

Zipitis, C.S. and Akobeng, A.K., 2008. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Archives of Disease in Childhood 93, 512-517.

Zittermann, A., Iodice, S., Pilz, S., Grant, W.B., Bagnardi, V. and Gandini, S., 2012. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. American Journal of Clinical Nutrition 95, 91-100.

Key facts

- The estimated worldwide prevalence of vitamin D deficiency among the elderly is of about 50%.
- Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia.
- Low vitamin D3 has been associated with significant reduction of mortality in older persons.
- A cycle for increasing risk for falling in older persons with low vitamin D has been hypothesized and reported.
- Vitamin D supplementation should be performed daily with at least 1000 UI.

Summary points

- The estimated worldwide prevalence of vitamin D deficiency among the elderly is of about 50%. Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia.
- The Institute of Medicine has recently recommended that serum 25-hydroxyvitamin D is adequate when it is higher than 50 nmol/l, which is similar to the recommendation of the Standing Committee of Europe Doctors (www.cpme.eu).
- A recent Cochrane review studying the relationship between vitamin-D and mortality reported that when the different forms of vitamin D are assessed separately, only low vitamin D3 levels significantly predict mortality, whereas vitamin D2, alfacalcidol, or calcitriol do not.
- The mechanism by which vitamin D deficiency could influence the muscle-skeletal system and can produce an increased risk for falling is described. We propose in older persons a vicious circle induced by low vitamin-D. Sarcopenia, altered cortical bone, and subsequent falls all contribute to osteoporotic fractures.
- Supplementation with cholecalciferol 1000 IU per day still remains the treatment of choice of vitamin D supplementation.

2. Vitamin D in health of seniors

F. Lauretani[1], M. Maggio[2], C. Ruggiero[3], G.P. Ceda[2] and L. Ferrucci[4]
[1]Geriatric Unit, Laboratory of Movement Analysis, Geriatric-Rehabilitation Department, University Hospital of Parma, Via Gramsci 14, 43126 Parma, Italy; [2]Department of Internal Medicine and Biomedical Sciences, Section of Geriatrics, University of Parma, Via Gramsci 14, 43126 Parma, Italy; [3]Institute of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, University of Perugia, S. Andrea delle Fratte, 06156 Perugia, Italy; [4]Longitudinal Studies Section, Clinical Research Branch, National Institute on Aging, 2122 S. Hanover Street, Baltimore, MD 53001, USA; flauretani@ao.pr.it

Abstract

Vitamin D is a hormone with multiple biological activities that are essential for normal functioning of many biological systems including bone, skeletal muscle, brain and heart. The estimated worldwide prevalence of vitamin D deficiency among the elderly is of about 50% and, because of the demographic expansion, vitamin D deficiency is becoming very important for public health. Vitamin D status has been studied in all continents and most countries over the world. Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia. In this chapter, we explore potential mechanism which vitamin D could influence the muscle-skeletal system and increase risk of falling. In particular, we propose an array of mechanisms for the increased risk of falling secondary to low vitamin D. These mechanisms can be directly or indirectly related to sarcopenia (defined as reduced muscle mass with age in older persons) – and are implicated in the genesis of osteoporotic fractures. Although we mention all therapeutic options for treatment of vitamin D deficiency, we recognize that in a recent Cochrane review addressing the relationship between vitamin D and mortality, only low vitamin D3 levels were significantly associated with high risk of death whereas vitamin D2, alfacalcidol, or calcitriol were not. Therefore, the treatment of choice for vitamin D deficiency should be cholecalciferol, also known as vitamin D3. Supplementation with 1000 IU per day still remains the treatment of choice of vitamin D supplementation.

Keywords*:* vitamin D deficiency, mortality, sarcopenia, falls, older persons

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_2, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
BMD	Bone mineral density
CI	Confidence interval
OR	Odds ratio
RR	Relative risk
VDR	Vitamin D receptor

2.1 Epidemiology of the vitamin-D deficiency

Older persons are prone to develop low vitamin D levels. This phenomenon results from reduced capacity of the skin to produce vitamin D, low sun exposure, skin pigmentation, sunscreen use, skin covering clothes and a diet low in fish and dairy products. In the elderly the reduced dermal synthesis of vitamin D is unlikely to be compensated by dietary intake of vitamin D. The estimated worldwide prevalence of vitamin D deficiencyamong the elderly of about 50% underlines the importance of vitamin D deficiency for public health (Lauretani *et al.*, 2010). Vitamin D status has been studied in all continents and most countries over the world. Vitamin D deficiency in adults is highly prevalent especially in the Middle-East and Asia (Ahmadieh and Arabi, 2011; Arabi *et al.*, 2010; Van Schoor *et al.*, 2011). Traditional risk groups include young children, pregnant women, and in older persons, the institutionalized and non-western immigrants.

The best determinant of vitamin D status is the serum concentration of 25(OH)D. There is no general agreement on the required serum 25(OH)D for an adequate vitamin D status. Most investigators agree that serum 25(OH)D should be higher than 50 nmol/l, but some recommends higher serum levels, e.g. higher than 75 or even 100 nmol/l (Holick, 2007). The Institute of Medicine has recently recommended that serum 25(OH)D is adequate when the serum concentration is higher than 50 nmol/l (Ross *et al.*, 2011). This statement is similar to the recommendation of the Standing Committee of Europe Doctors (www.cpme.eu). Clinical vitamin D deficiency only occurs when serum 25(OH)D is lower than 25 nmol/l. The clinical picture includes muscle weakness, bone pain and fractures (Ahmadieh and Arabi, 2011; Van Arabi *et al.*, 2010; Schoor *et al.*, 2011).

In a study of community dwelling persons, representative of the older persons, performed in the Chianti area in the centre of the Italy, known for its temperate climate and sunny countryside, a high prevalence of low 25(OH)D levels was found (Figure 2.1) (Maggio *et al.*, 2005). Serum levels of vitamin D diminish with age in both sexes, but the decline starts substantially earlier and is steeper in women starting from the perimenopausal period. In men the decline of vitamin D becomes apparent 20 years later starting from 7^{th} decade (Maggio *et al.*, 2005). In another study conducted in a US cohort of older men in the United States, both vitamin D deficiency and insufficiency were common. Approximately one fourth had 25(OH)D levels below the threshold

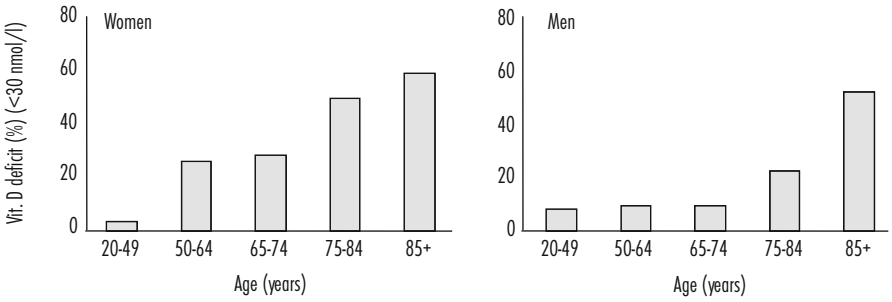

Figure 2.1. Prevalence of vitamin D deficiency in the InCHIANTI study population.

of frank deficiency (<20 ng/ml), and the majority had vitamin D insufficiency (<30 ng/ml) (Orwoll *et al.*, 2009). Vitamin D deficiency was particularly common during the winter and spring time (especially in the northern communities) and in the oldest and more obese subjects. In fact, 86% of these subjects with multiple risk factors were vitamin D deficient (Orwoll *et al.*, 2009).

2.2 Vitamin D and mortality in older persons

The relationship between vitamin D and mortality was recently reviewed in the Cochrane (Bjelakovic *et al.*, 2011) review of fifty randomized trials conducted in 94,148 participants with data available on mortality. Most of the trials included 70 years or older women. Vitamin D was administered for a median of two years. More than one half of the trials had a low risk of bias. Overall, vitamin D decreased mortality (RR 0.97, 95% CI = 0.94 to 1.00). When the different forms of vitamin D were assessed separately, only vitamin D3 decreased mortality significantly (RR 0.94, 95% CI = 0.91 to 0.98; 74,789 participants, 32 trials) whereas vitamin D2, alfacalcidol, or calcitriol did not. Vitamin D3 combined with calcium increased the risk of nephrolithiasis (RR 1.17, 95% CI = 1.02 to 1.34). Alfacalcidol and calcitriol increased the risk of hypercalcaemia (RR 3.18, 95% CI = 1.17 to 8.68).

In accordance with these results, Heaney *et al.* (2011) recently published a trial comparing the supplementation of D2 or D3 on incremental value of total 25(OH)D. The trial used a single-blind, randomized design in 33 healthy adults. Calciferols were dosed at 50,000 IU/wk for 12 wk. The authors found that D3 was approximately 87% more potent in raising and maintaining serum 25(OH)D concentrations and produces 2- to 3-fold greater storage of vitamin D than does equimolar D2. Hence they concluded that given its greater potency and lower cost, D3 should be the preferred treatment option when correcting vitamin D deficiency.

Established causes of increased mortality due to vitamin D deficiency are cardiovascular events (McGreevy and Williams, 2011), such as to myocardial dysfunction (Semba *et al.*, 2010), insulin-resistance (Liu *et al.*, 2009), arterial hypertension (Dobnig *et al.*, 2008) and heart failure (Pilz *et*

al., 2008). Experimental studies have also suggested that vitamin D plays a role in the regulation of several important inflammatory cytokines (such as IL-6 and TNF-alpha) (Nemerovski *et al.*, 2009). This mechanism could explain why vitamin D deficiency has been linked to several types of cancer (prostate, breast and colon cancer) (Chung *et al.*, 2011; Yin *et al.*, 2009) and infection diseases (Lehouck *et al.*, 2012; Yamshchikov *et al.*, 2009).

Recently, the presence of vitamin D receptor and the vitamin D activating enzyme, 1-hydroxylase, in the brain has suggested a potential beneficial role of vitamin D in cognitive function (Miller, 2010). In details, the vitamin D receptor and catalytic enzymes are localized in the areas of the brain involved in complex planning, processing, and the formation of new memories. These findings potentially implicate the role of vitamin D in cognitive impairment (Llewellyn *et al.*, 2010), depression (Milaneschi *et al.*, 2010) and also multiple sclerosis (Soilu-Hänninen *et al.*, 2008).

2.3 Vitamin D and falls in older persons

Falls in older persons are a public-health problem because of the high prevalence (about 30% among subjects aged 65 or older) and because they often result in adverse outcomes (Annweiler *et al.*, 2010). In particular, fall-related fractures are associated with higher morbidity and mortality, and substantial financial cost (Tinetti *et al.*, 2008). In order to delay the occurrence of falls and to reduce its individual and public health impact, effective preventive interventions and strategies need be identified. Data accumulated since the original publication by Chapuy *et al.* (1992) on the effects of vitamin D supplementation showed, despite several negative results (Grant *et al.*, 2005; Porthouse *et al.*, 2005), a reduction of fall and bone fracture rates (Chung *et al.*, 2011; Gillespie *et al.*, 2009). As a consequence, a vitamin D-calcium supplementation appears to be a low-cost and effective strategy for the reduction of fall and fracture (Bischoff-Ferrari *et al.*, 2003, 2004a, 2009; Chung *et al.*, 2011).

The mechanism by which low vitamin D could negatively influence the muscular-skeletal system producing an increased risk for falling is shown in Figure 2.2. We proposed in older persons a vicious circle for the risk of falling induced by vitamin D, sarcopenia (defined as reduced muscle mass with age in older persons) and altered cortical bone, as the main mediators of the final event represented by osteoporotic fractures (Figure 2.3).

Several studies clearly reported that an impairment of the cortical bone is the main factor responsible of the development of osteoporotic fractures (Dempster *et al.*, 1994; Mayhew *et al.*, 2005; Seeman and Delmas, 2006; Zebaze *et al.*, 2010). There is evidence that most of bone loss occurs after the age of 60 and is cortical, rather than trabecular, at peripheral sites (Seeman and Delmas, 2006). About 50% of cortical bone loss at peripheral sites is the result of remodeling within the cortex adjacent to the marrow (Zebaze *et al.*, 2010).

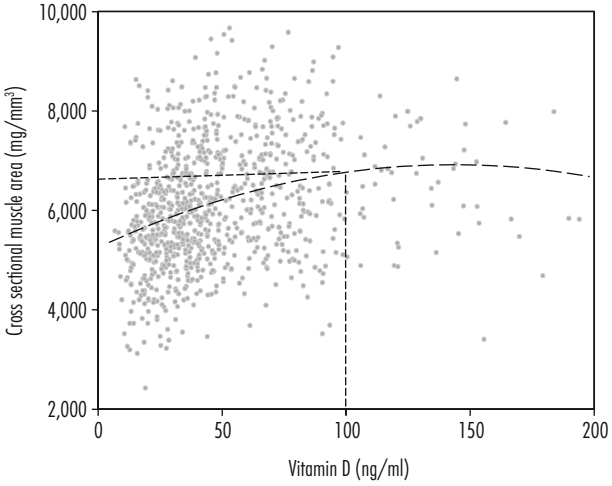

Figure 2.2. Relationship between vitamin D and muscle mass in older persons.

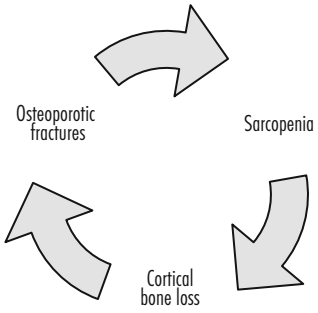

Figure 2.3. Spirality of falls vitamin D: deficiency induced in older persons considering musculo-skeletal system.

We previously showed, using data from the InCHIANTI, a representative study of the older persons living in the community, that calciotrophic hormones were strong independent correlates of cortical bone characteristics in women, being 25(OH)D positively associated with cortical bone area and volumetric cortical bone density (Figure 2.4) (Lauretani *et al.*, 2006). We found a clear dose-effect in the relationship between cortical bone density and serum level of vitamin D in older women, which is maximal below 30 nmol/l.

Both trabecular and cortical bone density were lower in old age in both sexes; while changes in bone geometry were substantially different in two sexes (Russo *et al.*, 2006). In men, cortical density but not cortical area was lower in old age, with a significant increase in bone size. In women, both cortical bone area and density were lower in old age, but the increase in total bone area was less pronounced than in men (Lauretani *et al.*, 2008). Factors known to be associated with increased bone resorption (such as 25(OH)D) mainly affected volumetric BMD, while others

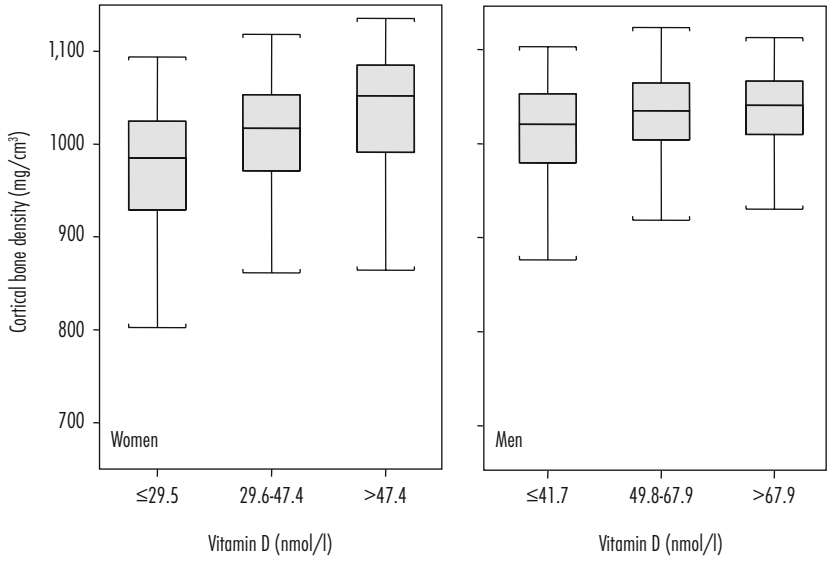

Figure 2.4. Relationship between vitamin D and cortical bone density.

associated with delivering mechanical forces on bone such as muscle mass primarily modulated bone geometry, producing bone fragility and subsequent falls from osteoporotic fractures in non-vertebral sites (Lauretani *et al.*, 2006). Since these physiological factors are potentially modulated by different preventive or pharmacological treatment of osteoporosis, these data may help to predict which bone site can be affected by a particular intervention. For instance, based on our data, we may hypothesize that an increase in muscle mass obtained by means of progressive resistance training will have a greater effect on bone geometry parameters rather than on volumetric BMD. Similarly, vitamin D compounds or teriparatide will be more effective in modulating directly vBMD rather than geometric parameters.

2.3.1 Examining effects of vitamin D on bone

Vitamin D deficiency has both direct and indirect consequences on bone cell function (Holick, 2007). Direct effects mainly concern reduced recruitment and differentiation of osteoclastic progenitors into mature osteoclasts, mostly mediated by osteoblast secretion of activating factors. There is also a diminished synthesis of specific collagenous and noncollagenous proteins in the osteoblasts. The main indirect consequences of vitamin D deficiency on bone cell function are defective mineralization of osteoid seams, due to inadequate intestinal absorption of calcium and phosphate, and an age-related form of compensatory hyperparathyroidism, which drives an accelerated bone loss. We suggested that the minimal 25(OH)D serum concentrations needed to avoid compensatory hyperparathyroidism are significantly higher at older ages (Maggio *et al.*, 2005). In a prospective, observational study designed to analyze risk factors for fracture in an ambulatory population aged >55 years, 73 (88%) had evidence of osteopenia or osteoporosis (T-score <-1.5) and/or low 25(OH)D (Seton *et al.*, 2005). Similar results were observed in patients

enrolled in two Finnish hospitals for fracture during approximately 13 months with hip fracture, fresh of previous events (Nurmi *et al.*, 2005).

Recent data from the Osteoporotic Fractures in Men (MrOS) study performed in healthy older men and focusing on osteoporosis showed that 25(OH)D level <20 ng/ml is associated with greater rates of hip bone loss, while rates of bone loss were similar among men with higher levels of total 25(OH)D (Ensrud *et al.*, 2009). The association between 25(OH)D levels and bone loss was stronger among men 75 years and older (Ensrud *et al.*, 2009). These findings suggest that low 25(OH)D levels are detrimental to BMD in older men.

2.3.2 Examining effects of vitamin D on skeletal muscle

Muscle weakness has long been associated with vitamin D deficiency. A vitamin D receptor is present in skeletal muscle (Simpson *et al.*, 1985), and vitamin D deficiency has been associated with proximal muscle weakness, increase in body sway, and an increased risk of falling (Dam *et al.*, 2009).

Vitamin D deficiency in adults can also cause a skeletal mineralization defect. The non-mineralized osteoid provides little structural support for the periosteal covering. As a result, patients with osteomalacia often complain of isolated or global bone discomfort along with aches and pains in their joints and muscles (Hicks *et al.*, 2008). These patients may be misdiagnosed with fibromyalgia, dysthymia, degenerative joint disease, arthritis, chronic fatigue syndrome, and other diseases (Plotnikoff and Quigley, 2003).

Speed performance and proximal muscle strength were markedly improved when 25(OH)D levels increased from 4 to 16 ng/ml (10 to 40 nmol/l) and continued to improve as the levels increased to more than 40 ng/ml (100 nmol/l) (Bischoff-Ferrari *et al.*, 2004b). Interestingly, persons with low (<25 nmol/l) baseline 25(OH)D levels were 2.57 (95% confidence interval 1.40-4.70, based on grip strength) and 2.14 (0.73-6.33, based on muscle mass) times more likely to experience sarcopenia, compared with those with high (>50 nmol/l) levels (Visser *et al.*, 2003). These results are in accordance with data reported in Figure 2.3. Using data from the InChianti study, we found a direct correlation between the cross-sectional muscle area – which is a proxy of the muscle mass – of the lower leg and the serum levels of vitamin D from 0 to 100 nmol/l. Thereafter, this correlation was lost, probably because of achievement of the plateau effect on the vitamin D on muscle mass.

In a previous cross-sectional analysis conducted at baseline in the InCHIANTI study (Houston *et al.*, 2007; Shardell *et al.*, 2009) low serum 25(OH)D levels was associated with impaired physical performance. Low 25(OH)D may affect physical performance and frailty, defined as 'a biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems and causing vulnerability to adverse outcomes', via effects on muscle strength. VDRs are located in skeletal muscle cells, and low 25(OH)D may result in decreased muscle strength from both decreased muscle synthesis and altered contractile

properties of muscle. Muscle protein synthesis is initiated by binding $1,25(OH)_2D$ to its nuclear receptor. The influence of $1,25(OH)_2D$ on calcium homeostasis is believed to influence contractile properties of muscle cells via both a VDR-mediated genomic pathway and a non genomic rapid mechanism. Thus, the association between low 25(OH)D and frailty may be explained by associations of insufficient 25(OH)D with sarcopenia and muscle weakness because both exert a central role in the development of the frailty syndrome. In a cross-sectional study, 6 minute walk distance was correlated with higher 25(OH)D) level even in patients affected by chronic heart disease (Boxer *et al.*, 2008). In the Longitudinal Aging Study Amsterdam, an association between vitamin D and physical function has been described. Compared with individuals with serum 25(OH)D levels above 30 ng/ml, physical performance was poorer in participants with serum 25(OH)D less than 10 ng/ml [regression coefficient (B) = -1.69; 95% CI = -2.28 to -1.10], and with serum 25(OH)D of 10-20 ng/ml (B = -0.46; 95% CI = -0.90 to -0.03). After adjustment for confounding variables, participants with 25(OH)D less than 10 ng/ml and 25(OH)D between 10 and 20 ng/ml had significantly higher OR for 3-year decline in physical performance (OR=2.21; 95% CI=1.00-4.87; and OR=2.01; 95% CI=1.06-3.81), compared with participants with 25(OH)D of at least 30 ng/ml (Wicherts *et al.*, 2007).

In a meta-analysis recently published (Annweiler *et al.*, 2009), this relationship was further investigated. Of the 102 selected studies, 16 met the selection criteria and were included in the final analysis. There were 8 observational studies and 8 intervention studies. The number of participants ranged from 24 to 33,067. A majority of studies examined community-dwelling older women. Five observational studies showed a significant positive association, whereas three studies did not. Four of the 5 studies and two of the 3 studies which tested the vitamin D supplementation effect, respectively on balance and gait, showed no significant effect. Four studies showed a significant effect on muscle strength, while this effect was not observed in three other studies. In addition, there was no significant association between vitamin D supplementation and an improvement of the sit-to-stand test in 50% of the studies. Authors concluded that, the association between vitamin D and physical performance remains controversial. Observational studies and clinical trials yielded divergent results, which highlights the complex and to date still poorly understood association between serum vitamin D concentration or vitamin D supplementation and physical performance (Annweiler *et al.*, 2009).

2.4 Options for vitamin D therapy in older persons

The treatment of choice for vitamin D deficiency is vitamin D, cholecalciferol, also known as vitamin D3. Cholecalciferol is available in 400, 1000, 2,000, 5,000, 10,000, 50,000 and even 300,000 UI capsules. Supplementation with 1000 IU per day will usually result in about a 10 ng/ml elevation of serum 25(OH)D when given over 3-4 months. Formulation of vitamin D3 of 300,000 IU is also present as intramuscular options, and given 300,000 IU annually corresponds to about 800 IU daily. A recent double-blind, placebo-controlled trial of 2,256 community-dwelling women, aged 70 years or older, considered to be at high risk of fracture were randomly assigned to receive cholecalciferol (a single annual dose of 500,000 IU of cholecalciferol administered orally)

or placebo each autumn to winter for 3 to 5 years. Contrary of their hypothesis, they found that participants receiving annual high-dose oral cholecalciferol experienced 15% more falls and 26% more fractures than the placebo group (Sanders *et al.*, 2010). Therefore, supplementation with 1,500-2,000 (Pramyothin and Holick, 2012; Wimalawansa, 2012) IU per day still remains the treatment of choice for vitamin D supplementation. Specific conditions are:

- Primary and secondary prevention for bone loss – cholecalciferol 1000 IU/day plus calcium 1 g/day.
- Inflammatory Bowel Diseases (IBD) – cholecalciferol 1000 IU/day plus calcium 1 g/day.
- Liver diseases – calcifediol (25(OH)D3) or 1-alpha (OH) calcidiol.
- Kidney diseases – calcitriol 0.5 μg/day plus calcium 1 g/day.

In conclusion, vitamin D is a hormone with several actions, and is fundamental for many biological systems including cortical bone and skeletal muscle.

Recent studies suggest that assessment of Vitamin D status should be recommended not only for prevention and treatment of osteoporosis but also included in the global evaluation of risk of falling, sarcopenia, neurological diseases, cardiovascular disease and cancer especially in older populations.

We should always consider that low vitamin D observed during chronic disease (such as diabetes and heart failure) could be a surrogate marker rather than be a causal mechanism. In addition because it is a hormone, vitamin D replacement in older people should be carefully monitored for avoiding side-effects of the supplementation such as nephrolithiasis.

References

Ahmadieh, H. and Arabi, A., 2011. Vitamins and bone health: beyond calcium and vitamin D. Nutrition Reviews 69, 584-598.

Annweiler, C., Montero-Odasso, M., Schott, A.M., Berrut, G., Fantino, B. and Beauchet, O., 2010. Fall prevention and vitamin D in the elderly: an overview of the key role of the non-bone effects. Journal of NeuroEngineering and Rehabilitation 11, 7-50.

Annweiler, C., Schott, A., Berrut, G., Fantino, B. and Beauchet, O., 2009. Vitamin d-related changes in physical performance: a systematic review. Journal of Nutrition, Health and Aging 13, 893-898.

Arabi, A., El Rassi, R. and El-Hajj Fuleihan, G., 2010. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nature Reviews Endocrinology 6, 550-561.

Bischoff-Ferrari, H.A., Dawson-Hughes, B, Staehelin, H.B., Orav, J.E., Stuck, A.E., Theiler, R., Wong, J.B., Egli, A., Kiel, D.P. and Henschkowski, J., 2009. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. British Medical Journal 339, b3692.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Willett, W.C., Staehelin, H.B., Bazemore, M.G., Zee, R.Y. and Wong, J.B., 2004a. Effect of vitamin D on falls. Journal of the American Medical Association 291, 1999-2006.

Bischoff-Ferrari, H.A., Dietrich, T., Orav, E.J., Hu, F.B., Zhang, Y., Karlson, E.W. and Dawson-Hughes, B., 2004b. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged ≥60 y. American Journal of Clinical Nutrition 80, 752-758.

Bischoff-Ferrari, H.A., Staehelin, H.B., Dick, W., Akos, R., Knecht, M., Salis, C., Nebiker, M., Theiler, R., Pfeifer, M., Begerow, B., Lew, R.A. and Conzelmann, M., 2003. Effect of vitamin D and calcium supplementation on falls: A randomized controlled trial. Journal of Bone and Mineral Research 18, 343-351.

Bjelakovic, G., Gluud, L.L., Nikolova, D., Whitfield, K., Wetterslev, J., Simonetti, R.G., Bjelakovic, M. and Gluud, C., 2011. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database of Systematic Reviews 6, CD007470.

Boxer, R.S., Dauser, D.A., Walsh, S.J., Hager, W.D. and Kenny, A.M., 2008. The association between vitamin D and inflammation with the 6-minute walk and frailty in patients with heart failure. Journal of the American Geriatrics Society 56, 454-461.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas P.D. and Meunier, P.J., 1992. Vitamin D3 and calcium to prevent hip fractures in the elderly women. New England Journal of Medicine 327, 1637-1642.

Chung, M., Lee, J., Terasawa, T., Lau, J. and Trikalinos, T.A., 2011. Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the US Preventive Services Task Force. Annals of Internal Medicine 155, 827-838.

Dam, T.T., von Mühlen, D. and Barrett-Connor, E.L., 2009. Sex-specific association of serum vitamin D levels with physical function in older adults. Osteoporosis International 20, 751-760.

Dempster, D.W., Parisien, M., Silverberg, S.J., Liang, X.G., Schnitzer, M., Shen, V., Shane, E., Kimmel, D.B., Recker, R., Lindsay, R. and Bilezikian, J.P., 1999. On the mechanism of cancellous bone preservation in postmenopausal women with mild primary hyperparathyroidism. Journal of Clinical Endocrinology and Metabolism 84, 1562-1566.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., Kinkeldei, J., Boehm, B.O., Weihrauch, G. and Maerz, W., 2008. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Archives of Internal Medicine 168, 1340-1349.

Ensrud, K.E., Taylor, B.C., Paudel, M.L., Cauley, J.A., Cawthon, P.M., Cummings, S.R., Fink, H.A., Barrett-Connor, E., Zmuda, J.M., Shikany, J.M., Orwoll, E.S. and Osteoporotic Fractures in Men Study Group, 2009. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. Journal of Clinical Endocrinology and Metabolism 94, 2773-2780.

Gillespie, L.D., Robertson, M.C., Gillespie, W.J., Lamb, S.E., Gates, S., Cumming, R.G. and Rowe, B.H., 2009. Interventions for preventing falls in older people living in the community. Cochrane Database of Systematic Reviews 15, CD007146.

Grant, A.M., Avenell, A., Campbell, M.K., McDonald, A.M., MacLennan, G.S., McPherson, G.C., Anderson, F.H., Cooper, C., Francis, R.M., Donaldson, C., Gillespie, W.J., Robinson, C.M., Torgerson, D.J., Wallace, W.A. and RECORD Trial Group, 2005. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (randomized evaluation of calcium or vitamin D, RECORD): a randomized placebo-control trial. Lancet 365, 1621-1628.

Heaney, R.P., Recker, R.R., Grote, J., Horst, R.L. and Armas, L.A., 2011. Vitamin D(3) is more potent than vitamin D(2) in humans. Journal of Clinical Endocrinology and Metabolism 96, E447-52.

Hicks, G.E., Shardell, M., Miller, R.R., Bandinelli, S., Guralnik, J., Cherubini, A., Lauretani, F. and Ferrucci, L., 2008. Associations between vitamin D status and pain in older adults: the Invecchiare in Chianti study. Journal of the American Geriatrics Society 56, 785-791.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Houston, D.K., Cesari, M., Ferrucci, L., Cherubini, A., Maggio, D., Bartali, B., Johnson, M.A., Schwartz, G.G. and Kritchevsky, S.B., 2007. Association between vitamin D status and physical performance: the InCHIANTI study. Journal of Gerontology: Medical Sciences 62, 440-446.

Lauretani, F., Bandinelli, S., Russo, C.R., Maggio, M., Di Iorio, A., Cherubini, A., Maggio, D., Ceda, G.P., Valenti, G., Guralnik, J.M. and Ferrucci, L., 2006. Correlates of bone quality in older persons. Bone 39, 915-921.

Lauretani, F., Maggio, M., Valenti, G., Dall'Aglio, E. and Ceda, G.P., 2010. Vitamin D in older population: new roles for this 'classic actor'? Aging Male 13, 215-232.

Lauretani. F., Bandinelli. S., Griswold, M.E., Maggio, M., Semba, R., Guralnik, J.M. and Ferrucci, L., 2008. Longitudinal changes in BMD and bone geometry in a population-based study. Journal of Bone and Mineral Research 23, 400-408.

Lehouck, A., Mathieu, C., Carremans, C., Baeke, F., Verhaegen, J., Van Eldere, J., Decallonne, B., Bouillon, R., Decramer, M. and Janssens, W., 2012. High doses of vitamin d to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. Annals of Internal Medicine 156, 105-114.

Liu, E., Meigs, J.B., Pittas, A.G., McKeown, N.M., Economos, C.D., Booth, S.L. and Jacques, P.F., 2009. Plasma 25-hydroxyvitamin d is associated with markers of the insulin resistant phenotype in nondiabetic adults. Journal of Nutrition 139, 329-334.

Llewellyn, D.J., Lang, I.A., Langa, K.M., Muniz-Terrera, G., Phillips, C.L., Cherubini, A., Ferrucci, L. and Melzer, D., 2010. Vitamin D and risk of cognitive decline in elderly persons. Archives of Internal Medicine 170, 1135-1141.

Maggio, D., Cherubini, A., Lauretani, F., Russo, R.C., Bartali, B., Pierandrei, M., Ruggiero, C., Macchiarulo, M.C., Giorgino, R., Minisola, S. and Ferrucci, L., 2005. 25(OH)D Serum levels decline with age earlier in women than in men and less efficiently prevent compensatory hyperparathyroidism in older adults. Journal of Gerontology: Medical Sciences 60, 1414-1419.

Mayhew, P.M., Thomas, C.D., Clement, J.G., Loveridge, N., Beck, T.J., Bonfield, W., Burgoyne, C.J. and Reeve, J., 2005. Relation between age, femoral neck cortical stability, and hip fracture risk. Lancet 366, 129-135.

McGreevy, C. and Williams, D., 2011. New insights about vitamin d and cardiovascular disease: a narrative review. Annals of Internal Medicine 155, 820-826.

Milaneschi, Y., Shardell, M., Corsi, A.M., Vazzana, R., Bandinelli, S., Guralnik, J.M. and Ferrucci, L., 2010. Serum 25-hydroxyvitamin D and depressive symptoms in older women and men. Journal of Clinical Endocrinology and Metabolism 95, 3225-3233.

Miller, J.W., 2010. Vitamin D and cognitive function in older adults: are we concerned about vitamin D-mentia? Neurology 74, 13-15.

Nemerovski, C.W., Dorsch, M.P., Simpson, R.U., Bone, H.G., Aaronson, K.D. and Bleske, B.E., 2009. Vitamin D and cardiovascular disease. Pharmacotherapy 29, 691-708.

Nurmi, I., Kaukonen, J.P., Lüthje, P., Naboulsi, H., Tanninen, S., Kataja, M., Kallio, M.L. and Leppilampi, M., 2005. Half of the patients with an acute hip fracture suffer from hypovitaminosis D: a prospective study in south eastern Finland. Osteoporosis International 16, 2018-2024.

Orwoll, E., Nielson, C.M., Marshall, L.M., Lambert, L., Holton, K.F., Hoffman, A.R., Barrett-Connor, E., Shikany, J.M., Dam, T., Cauley, J.A. and Osteoporotic Fractures in Men (MrOS) Study Group, 2009. Osteoporotic Fractures in Men (MrOS) Study Group. Vitamin D deficiency in older men. Journal of Clinical Endocrinology and Metabolism 94, 1214-1222.

Pilz, S., März, W., Wellnitz, B., Seelhorst, U., Fahrleitner-Pammer, A., Dimai, H.P., Boehm, B.O. and Dobnig, H., 2008. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. Journal of Clinical Endocrinology and Metabolism 93, 3927-3935.

Plotnikoff, G.A. and Quigley, J.M., 2003. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clinic Proceedings 78, 1463-1470.

Porthouse, J., Cockayne, S., King, C., Saxon, L., Steele, E., Aspray, T., Baverstock, M., Birks, Y., Dumville, J., Francis, R., Iglesias, C., Puffer, S., Sutcliffe, A., Watt, I. and Torgerson, D.J., 2005. Randomized controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. British Medical Journal 330, 1-6.

Pramyothin, P. and Holick, M.F., 2012. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. Current Opinion in Gastroenterology 28, 139-150.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. Journal of Clinical Endocrinology and Metabolism 96, 53-58.

Russo, C.R., Lauretani, F., Seeman, E., Bartali, B., Bandinelli, S., Di Iorio, A., Guralnik, J. and Ferrucci, L., 2006. Structural adaptations to bone loss in aging men and women. Bone 38, 112-118.

Sanders, K.M., Stuart, A.L., Williamson, E.J., Simpson, J.A., Kotowicz, M.A., Young, D. and Nicholson, G.C., 2010. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. Journal of the American Medical Association 303, 1815-1822.

Seeman, E. and Delmas, P.D., 2006 Bone quality – the material and structural basis of bone strength and fragility. New England Journal of Medicine 354, 2250-2261.

Semba, R.D., Houston, D.K., Bandinelli, S., Sun, K., Cherubini, A., Cappola, A.R., Guralnik, J.M. and Ferrucci, L., 2010. Relationship of 25-hydroxyvitamin D with all-cause and cardiovascular disease mortality in older community-dwelling adults. European Journal of Clinical Nutrition 64, 203-209.

Seton, M., Jackson, V., Lasser, K.E., Doppelt, S., Pierre-Jacques, M. and Connelly, M., 2005. Low 25-hydroxyvitamin D and osteopenia are prevalent in persons ≥55 yr with fracture at any site: a prospective, observational study of persons fracturing in the community. Journal of Clinical Densitometry 8, 454-460.

Shardell, M., Hicks, G.E., Miller, R.R., Kritchevsky, S., Andersen, D., Bandinelli, S., Cherubini, A. and Ferrucci, L., 2009. Association of low vitamin D levels with the frailty syndrome in men and women Journal of Gerontology: Medical Sciences 64, 69-75.

Simpson, R.U., Thomas, G.A. and Arnold, A.J., 1985. Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. Journal of Biological Chemistry 260, 8882-8891.

Soilu-Hänninen, M., Laaksonen, M., Laitinen, I., Erälinna, J.P., Lilius, E.M. and Mononen, I., 2008. A longitudinal study of serum 25-hydroxyvitamin D and intact parathyroid hormone levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis. Journal of Neurology, Psychiatry and Neurosurgery 79, 152-157.

Tinetti, M.E., Baker, D.I., King, M., Gottschalk, M., Murphy, T.E., Acampora, D., Carlin, B.P., Leo-Summers, L. and Allore, H.G., 2008. Effect of dissemination of evidence in reducing injuries from falls. New England Journal of Medicine 359, 252-261.

Van Schoor, N.M. and Lips, P., 2011. Worldwide vitamin D status. Best Practice & Research Clinical Endocrinology & Metabolism 25, 671-680.

Visser, M., Deeg, D.J., Lips, P. and Longitudinal Aging Study Amsterdam, 2003. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. Journal of Clinical Endocrinology & Metabolism 88, 5766-5772.

Wicherts, I.S., van Schoor, N.M., Boeke, A.J., Visser, M., Deeg, D.J., Smit, J., Knol, D.L. and Lips, P., 2007. Vitamin D status predicts physical performance and its decline in older persons. Journal of Clinical Endocrinology and Metabolism 92, 2058-2065.

Wimalawansa, S.J., 2012. Vitamin D in the New Millennium. Current Osteoporosis Reports 10, 4-15.

Yamshchikov, A.V., Desai, N.S., Blumberg, H.M., Ziegler, T.R. and Tangpricha, V., 2009. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocrine Practice 15, 438-449.

Yin, L., Grandi, N., Raum, E., Haug, U., Arndt, V. and Brenner, H., 2009. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. Alimentary Pharmacology and Therapeutics 30, 113-125.

Zebaze, R.M., Ghasem-Zadeh, A., Bohte, A., Iuliano-Burns, S., Mirams, M., Price, R.I., Mackie, E.J. and Seeman, E., 2010. Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. Lancet 375, 1729-1736.

Key facts

- A scientist received the Nobel Prize for isolating vitamin D and used cod liver oil to cure dogs with rickets.
- Vitamin D deficiency is a common condition but frequently seen in northern latitude countries.
- Sun exposure is the best way to increase vitamin D levels in the body.
- Vitamin D supplementation can improve vitamin D status drastically.
- A healthy vitamin D status can provide protection against many serious diseases such as rickets, osteomalacia, cancer and osteoporosis.

Summary points

- Vitamin D is an important micronutrient to healthy outcomes.
- Supplementation is necessary among vulnerable populations that may lack exposure to sunlight or possess risk factors.
- Dietary intake may be inadequate to maintain optimal vitamin D concentrations in the body.
- Several studies demonstrate increased supplementation leads to a healthy vitamin D status.
- Policies should incorporate a multi-strategic approach to supplementation at local and national levels.

3. Vitamin D supplementation and public health policy

R. Babaria and R.R. Watson
Mel and Enid Zuckerman College of Public Health, P.O. Box 245155, 1295 N. Martin, Tucson, AZ 85724-5155, USA; radhikababaria@gmail.com

Abstract

Vitamin D deficiency is a common condition among people throughout the world. It affects any age, gender, and ethnicity. However, certain individuals are at a greater risk due to a genetic predisposition or lifestyle. Sun exposure is the greatest source of vitamin D synthesis in the body. Often times, individuals may lack adequate exposure due to internal and external factors. Dietary food intake often is not sufficient to meet an adequate vitamin D status. Public health policies should target supplementation among vulnerable populations in order to decrease the prevalence of vitamin D deficiency. Successful public health strategies collaborate and network with various organizations dedicated to improving vitamin D status and incorporate a multi-strategic approach at both local and national levels.

Keywords: fortification, strategies, vulnerable populations, sunlight exposure

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_3, © Wageningen Academic Publishers 2013

Abbreviations

DRI	Dietary reference intake
IOM	North American Institute of Medicine
PA International	Public Advice International Foundation
serum 25(OH)D	Serum 25-hydroxyvitamin D
UVB	Ultraviolet B rays

3.1 Introduction

The significance of vitamin D in human health is evident since the early 20th century. Scientific researcher Elmer McCollum was the first to isolate vitamin D. McCollum received a Noble Prize for an experiment that used modified cod liver oil to cure dogs with rickets (Mohr, 2009). Scientists later discovered that the human body could synthesize vitamin D through UVB light exposure, giving the micronutrient the nickname, the 'sunshine vitamin'. In the 1930s, rickets was a major public health concern in the United States (ODS, 2011). A milk fortification program was a key method implemented to combat rickets and prevent a low vitamin D status.

According to the Center for Disease Control and Prevention, vitamin D deficiency is defined as a condition that causes 'inadequate mineralization or demineralization of the skeleton (CDC, 2008).' It is associated with rickets and osteomalacia in children and adults, respectively (Misra *et al.*, 2008). Vitamin D acts as a biological hormone within the body. By maintaining sufficient calcium and phosphate levels, adequate vitamin D concentrations promote bone and muscle health (ODS, 2011). Several studies associate vitamin D insufficiency to poor immune, respiratory, cardiovascular, cell proliferation, and metabolic functions; however, further research is necessary (Schwalfenberg and Genuis, 2010).

Determining a standard method to define and measure vitamin D status can improve the variability in data seen across countries. The best indicator to determine vitamin D status is serum 25(OH)D concentration (ODS, 2010). Although vitamin D deficiency is well-documented worldwide and numerous reports indicate the benefits from supplementation, there is a lack of supplementation policies and programs preventing and treating the health consequences due to this insufficiency.

3.2 Epidemiology

Vitamin D deficiency is common in Europe but varies greatly among European countries (Lips, 2007). Other regions with a high prevalence include the United States, Middle East, India, China and Japan (Holick and Chen, 2008). Vitamin D deficiency has no boundaries, affecting any age, gender, and ethnicity; however, certain populations are affected disproportionally. Children, elderly, and pregnant women are vulnerable subgroups for vitamin D deficiency. The prevalence

of vitamin D deficiency in the USA is especially common among minority groups. Data from the National Health and Nutrition Examination Survey conducted between 2005 and 2006 defined vitamin D deficiency as a serum 25(OH)D concentrations less than or equal to 20 ng/ml (50 nmol/l) (Forrest and Stuhldreher, 2011). The study found an overall 41.6% prevalence of vitamin D deficiency among the study participants (Forrest and Stuhldreher, 2011). Among the participants, 82.1% of the African Americans and 69.2% of the Hispanic were deficient (Forrest and Stuhldreher, 2011). Other risk factors for a poor vitamin D status include a lack of a college education, obesity, poor health status, and no daily milk consumption (Forrest and Stuhldreher, 2011). Skin pigmentation, gender, nutrition, lack of supplementation, and clothing style are also genetic and lifestyle risk factors (Lips, 2007).

3.3 Sources of vitamin D

Several sources of vitamin D include sun exposure, natural diet foods, fortified foods, and supplements (WHO, 2012). The best way to obtain vitamin D is sun exposure. Sun exposure enables UVB rays to reach exposed skin to produce vitamin D3. Factors that affect synthesis include the season, time of day, duration of exposure, pollution, skin pigmentation, and sunscreen use. Researchers recommend sun exposure of at least 15 to 30 minutes, twice a week (ODS, 2011). Example actions include increasing the amount of time exposed to sunlight or the type of clothing worn. Often times in countries that have a predominant religious association such as the Middle East, women are unable to alter clothing form. It becomes more crucial for these vulnerable populations to seek vitamin D through diet and supplementation. Individuals who have limited activity in sunlight should rely on diet and supplementation to benefit from optimal vitamin D effects.

Vitamin D, a fat-soluble micronutrient, is obtained from a limited number of naturally occurring foods. It is primarily found abundantly in fatty fish and eggs. Children are able to obtain vitamin D from fortified sources such as cereals, cheese, and milk. However, a vulnerable population includes darker skinned individuals lacking sufficient UVB exposure. Among this population, an emphasis on supplementation to meet adequate levels is crucial. Fortified foods are often inadequate to meet vitamin D requirements. In adults, diet alone constitutes only 10-20% of vitamin D concentrations (WHO, 2011). However, in the USA, fortified foods provide the most diet obtained vitamin D. In Canada, it is a law to fortify milk and margarine with vitamin D at 35 to 40 IU/100 ml and at or greater than 530 IU/100 g, respectively (ODS, 2011). It is also a law to fortify infant formula in the USA and Canada. The USA requires 40 to 100 IU/100 kcal vitamin D while Canada requires 40-80 IU/100 kcal (ODS, 2011). Taking into consideration a risk of inadequate sun exposure, children and adults may need at least 800-1000 IU vitamin D to maximize health benefits (Holick and Chen, 2008).

3.4 Public health strategies to maintain an adequate vitamin D status

Public health policies and strategies should target populations at risk for a compromised vitamin D status. Planned regular sun exposure is one of the best ways to synthesize vitamin D in the body but for public health policy purposes, the focus is on natural food sources, fortification, and supplementation.

3.4.1 Natural food sources

Very few naturally occurring foods are rich in vitamin D. Typically, animal foods contain cholecalciferol, while plant foods contain ergocalciferol. One study demonstrates that consuming fatty fish frequently is effective in maintaining serum 25(OH)D levels in elderly Japanese women (Nakamura *et al.*, 2000). Study participants who consumed fish more than four times a week had greater serum 25(OH)D levels than those women who ate fish less frequently or not at all. In addition, adequate serum 25(OH)D levels were maintained in the women who consumed fish more frequently during the winter. Liver meat is another source of abundant vitamin D. For vegetarians or vegans, all edible mushrooms have some content of vitamin D2 and ergosterol, which becomes activated with UVB exposure. Shitake mushrooms can have up to 25 μg/g vitamin D2 content (Nakamura *et al.*, 2000). Other mushroom types vary in vitamin D levels and optimal benefit is also contingent upon sunlight exposure.

One of the barriers documented in some studies is whether or not obtaining vitamin D through natural food sources is sufficient for an adequate vitamin D status. Recommendations for vitamin D vary between countries if provided at all. Estimating the amount of vitamin D intake is difficult because vitamin D content is not always accessible on food labels (Lamberg-Allardt, 2006). In Canada, nutritional content for vitamin D is generally available. Reporting vitamin D content on food products is one public health strategy that can facilitate public awareness towards conscious vitamin D intake.

3.4.2 Fortification

Fortification is one of the best methods to reach the general populations for vitamin D intake. It is a cost effective and feasible option for many foods in comparison to the treatment of problems that arise from vitamin D deficiency (Calvo and Whiting, 2006). One challenge in fortifying foods with vitamin D is that it may not be effective in meeting recommended levels for vitamin D intake. In the USA and Canada, fortifying milk levels are too low to increase or maintain serum 25(OH)D levels. A higher fortification as seen in powdered milk proved to be more effective in bone mineralization in women (Calvo and Whiting, 2006). Another limitation in fortification is that policies fortify select staple foods that may not reach all vulnerable risk groups or exclude individuals with specific dietary restrictions. A possible strategy to overcome this barrier is to increase the number and type of foods fortified, taking into consideration cultural preferences and accessibility.

3.4.3 Supplementation

When dietary intake of vitamin D is restricted or otherwise unavailable, supplementation is another option to help improve vitamin D status. Supplementation for vulnerable populations as a preventative strategy is more cost effective than screening for vitamin D status among all individuals. A challenge in supplementation strategies is compliance. In a European study assessing the vitamin D intakes in Denmark, Finland, Ireland, and Poland, use of supplements varied by country and age. Generally, only 38% of elderly women used supplements (Lamberg-Allardt, 2006). Of this, Denmark had the highest use while Poland had the lowest. In comparison, only 19% of young girls used supplements with Denmark again showing the highest use and Poland the least (Lamberg-Allardt, 2006). Similar variations are seen among racial and ethnic groups in the USA and Canada according to the National Health and Nutrition Examination Survey. When comparing African American and Caucasian women taking similar intake guidelines for vitamin D supplements, African Americans illustrated a greater prevalence of vitamin D deficiency. Markedly different vitamin D statuses among racial groups suggest a need to reflect racial and ethnic backgrounds in dietary guidelines. Another limitation on an individual basis is financial barriers in obtaining supplements. The best strategy would be to create evidence based supplementation programs for vulnerable populations in a community.

Data for vitamin D recommendations for infants, pregnant women, and adolescents is difficult to assess due to the lack of sufficient information (Cashman and Kiely, 2011). In Southeast China, a study conducted in the Hangzhou Zhejiang province demonstrated a very high prevalence of vitamin D deficiency among 1 month to 16 year olds (Zhu *et al.*, 2012). The study results recommend supplementing children through adolescence. The physiological mechanism of vitamin D during pregnancy and early infant development is not well known. Controversial findings of fetal skeletal development and adverse health outcomes such as low birth weight in infants and pre-eclampsia in mothers signify the need for further research in maternal vitamin D during pregnancy (Cashman and Kiely, 2011). In a systematic review, six randomized control trials examined vitamin D effects during pregnancy. Five trials tested vitamin D supplementation versus no supplementation or a placebo. Another trial compared vitamin D supplementation with calcium versus no supplementation. All trials demonstrated that supplementation increased serum 25(OH)D levels during pregnancy (De-Regil *et al.*, 2012). However, since there is no evidence of clinical benefits in preventing pregnancy or infant outcomes, WHO does not recommend vitamin D supplementation to prevent pre-eclampsia during pregnancy (WHO, 2011). Varying dosages, undetermined effects, and unknown safety of supplementation during pregnancy are a few examples that illustrate the need for guidance. With future research findings, evidence based public health strategies can be potentially made to establish recommended vitamin D supplementation levels for pregnant women (Cashman and Kiely, 2011).

3.5 Recommended supplementation

There are two physiologically important forms of vitamin D for supplementation: D2 and D3. These forms differ in the chemical side chain structures and both are used as nonprescription supplementation sources. In the USA, vitamin D2 is available through prescription (CDC, 2008). Vitamin D2, also known as ergocalciferol, is engineered by UV irradiation of ergosterols usually found in molds, yeasts, and higher order plants (CDC, 2008). Vitamin D3, also known as cholecalciferol, is synthesized by the UVB radiation of 7-dehydrocholesterol on the human skin (ODS, 2011). The National Institutes of Health compares the two forms of vitamin D supplementation and although firm conclusions about the different effects cannot be drawn, both forms increase serum 25(OH)D concentration (ODS, 2011). Ergocalciferol and cholecalciferol are biologically inert forms and both have similar mechanisms for metabolism in the human body (CDC, 2008). Vitamin D supplementation is often times mixed with calcium because vitamin D helps absorb calcium in the gut and maintain serum calcium and phosphate levels for bone mineralization (ODS, 2011).

In the USA, the IOM recommends dietary allowance of 600 IU per day for 1 to 70 years of age and over 20 IU per day for individuals older than 71 years of age (IOM, 2010). Pregnant and lactating women are recommended to take 600 IU of vitamin D per day (IOM, 2010). Adequate intake for infants is 400 IU per day (IOM, 2010). The tolerable upper intake level refers to the highest safe amount of vitamin D an individual can consume. The upper level intake for infants less than 6 months is 1000 IU per day and 1,500 IU per day for infants 6 months to 12 months of age (IOM, 2010). Children 1 to 3 years of age have an upper intake limit of 2,500 IU per day and children 4 to 8 years of age can have 3,000 IU per day (IOM, 2010). Individuals 9 to older than 71 years of age have an upper intake level of 4,000 IU per day along with pregnant and lactating women (IOM, 2010). Recommended dietary allowances for Canada are similar to that of the USA.

Based upon literature reviews, each institute in various countries has different recommendations for vitamin D supplementation and different definitions for vitamin D inadequacy. It is important to note that dietary intake of vitamin D can also vary among countries and individuals. For instance, Lamberg-Allardt (2006) discusses various studies to demonstrate that current dietary intake recommendations are too low to reach and maintain optimal serum 25(OH)D concentrations when sunlight exposure is absent. Supplementation results in increased serum 25(OH)D concentrations as exemplified in a study that involved two adolescent groups. One group was given a daily dose of 5 mg of vitamin D3 while the other group was given 10 mg for a total duration of 1 year (Lips, 2007). Comparing the two groups, the adolescents receiving 10 mg of vitamin D3 had greater vitamin D concentrations and maintained adequate levels from autumn to spring (Lips, 2007).

In Europe, the European Food Safety Authority adopted the upper intake values for vitamin D as recommended by the IOM. Representative data on vitamin D intake exists only for a limited number of European countries; however, the method for data collection using surveys and instruments is inconsistent and unreliable to make comparisons (Cashman and Kiely, 2011).

One of the challenges the DRI committee encountered in the 2010 review was assessing the dose-response relationship for vitamin D. It was a challenge to accurately determine sunlight exposure, and there was limited information on vitamin D status. Therefore, a key step in addressing vitamin D deficiency is quantifying the data to make comparisons among DRIs and vitamin D status in the population and addressing the gaps.

3.6 Supplemental toxicity

Vitamin D supplementation discussions usually bring a concern for toxicity. The upper intake limit for serum 25(OH)D concentrations is not established for various groups (Veith, 2007). Symptoms of toxicity include those of hypercalcemia, lethargy, dehydration, and nausea. One study states that sunlight can provide up to 10,000 IU per day if converted to the oral consumption equivalent (Veith, 2007). In comparison to the DRIs in the United States, this is a very large dose. Clinical trials conducted illustrate that current DRIs are very conservative and a prolonged intake of a higher dose such as 10,000 IU per day poses no adverse effect on health (Veith, 2007). Similar results are demonstrated in a study done by Hathcock *et al.* (2007). Although these intake levels seem alarming, the main purpose of presenting the finding is to exemplify the need for an established and uniform upper intake level to support supplementation. Overall, there is a low toxicity risk for supplemental use based on the current DRIs and fortification (Calvo and Whiting, 2006).

3.7 Global supplementation studies

3.7.1 North America

One possible method to target a vulnerable subgroup, such as the elderly, is to focus on controlled environments. An example of such an environment is institutional infrastructures. Now more than before, an increased number of seniors are utilizing nursing home facilities. A nursing home population is one of the groups susceptible to vitamin D insufficiency. Factors that lead this high risk of vitamin D deficiency include limited access to sunlight due to mobility issues, decreased vitamin D production with age. Individuals living at higher latitudes also have difficulty producing vitamin D skin synthesis due to weak UVB intensity. In a geriatric study, 68 nursing home patients were given 2,000 IU of daily vitamin D3 supplementation (Schwalfenberg and Genuis, 2010). Each patient involved in the retrospective chart review continued supplementation for at least five months. Approximately 94.1% of the patients improved their vitamin 25(OH)D status and none of the study residents reached toxicity (Schwalfenberg and Genuis, 2010). The study makes a strong recommendation to routinely supplement all nursing home residents daily unless otherwise noted for other health concerns. With this effort, vitamin D status and quality of life can be improved in nursing home patients and morbidity and mortality can be prevented (Schwalfenberg and Genuis, 2010).

The vitamin D initiative in the United States became more significant due to recent advances in vitamin D research over the past decade. The Office of Dietary Supplements at the National Institutes of Health coordinates the initiative. Key stakeholders and partners of the initiative include the US Department of Health and Human Services such as the Center for Disease Control and Prevention, National Institutes of Health, Food and Drug Administration, Agency for Healthcare Research and Quality, National Institute of Standards and Technology, US Department of Defense, US Department of Agriculture, and Health Canada (ODS, 2010). The Vitamin D Standardization Program began in 2010 as part of this initiative. The Office of Dietary Supplements at the National Institutes of Health aims to provide a standardized method of measuring vitamin D in foods and supplements and vitamin D status in the USA population. With standardized measurements, international efforts can expand to public health activities worldwide. The program includes national health survey participation from Australia, Canada, Germany, Ireland, Mexico, South Korea, the United Kingdom, and the USA. The representative method of data collection can provide a consistent manner for evaluation and comparison to inform public policy, research efforts, and guidelines (ODS, 2010).

3.7.2 Europe

In the European region, PA International is a key agency that focuses on health promotion and protection. More specifically, board members address vitamin D deficiency by collaborating with other organizations with missions that promote vitamin D health among populations (PA International, 2012). The stakeholders include Shine on Scotland, the Irish Osteoporosis Society, the Dutch Fall Prevention Network, and the German Osteoporosis Patient Society, and the Health Research Forum. According to PA International, one of the initial steps in addressing this issue is to collaborate between doctors, scientists, patients and representatives of politics, industry and the media. Further steps include developing policy briefs and increasing awareness of vitamin D deficiency as a public health concern. In 2010, WHO/Europe and PA International convened to address the vitamin D deficiency in Europe. WHO/Europe planned on developing an integrated strategy based on the Action Plan for Food and Nutrition Policy while PA International focused on fundraising opportunities. Through international collaboration and coordination of local stakeholders, vitamin D deficiency can be addressed more effectively (PA International, 2012).

A Finland study exemplifies how public policy to fortify specific foods with vitamin D can increase the levels of vitamin D status (Laaksi *et al.*, 2006). During the wintertime, vitamin D deficiency increases substantially among people in northern countries in response to decreased sun exposure; therefore, along with supplementation, fortification of vitamin D is another method to help increase vitamin D concentrations among populations. Finland exemplifies a successful fortification national public health policy based on the Ministry of Social Affairs and Health's recommendation. The policy targets fortifying liquid milk products, margarines, and butter with vitamin D across the country. Vitamin D status is determined using serum 25(OH)D as a marker in Finnish men aged 18 to 28 years (Laaksi *et al.*, 2006). Data on the vitamin D status of these men was collected in January 2003 and after the implementation of the policy in January 2004 (Laaksi *et al.*, 2006). Mean serum 25(OH)D concentrations increased by 50% after implementation

of the policy (Laaksi *et al.*, 2006). Creating a public policy to fortify standard food products with vitamin D is crucial in Europe as many countries vary in their mandatory and voluntary nutrient fortification requirements (Cashman and Kiely, 2011). In addition to supplementation, fortification is another method that can help public health efforts at a national level.

Ireland demonstrates how public health policy on vitamin D supplementation can target infants. The Food Safety Authority of Ireland recommends a universal vitamin D supplementation for all infants from birth to 1 year of age. The Department of Health and Children validated this evidence-based recommendation and has fulfilled responsibility to the Health Service Executive to implement the policy. More specifically, the policy states, '…all infants, from birth to 12 months, whether breastfed or formula fed, be given a daily supplement of 5 μg (200 IU) vitamin D. This should be provided by a supplement containing vitamin D exclusively (HSE, 2010).' According to the policy brief, 200 IU of vitamin D daily is sufficient to maintain serum 25(OH)D levels greater than 27.5 nmol/l (HSE, 2010). It is the responsibility of health professionals, community and hospital dietitians, practitioners, pharmacists, and other health care staff to inform parents about daily supplementation. It is important to note that the public health policy generalizes the population and parents of infants with specific medical conditions should seek medical advice from a physician. Between October to March, sunlight exposure is inadequate in the northern latitude of Ireland (HSE, 2010). Infants and young children are not recommended to seek direct UVB exposure and the infant diet is limited in vitamin D (HSE, 2010). These factors along with incidents of rickets in Ireland helped endorse the universal supplementation policy. The universal supplementation of 200 IU does not pose an issue for toxicity even if an infant is fed formula fortified with vitamin D (HSE, 2010). The public health implications of this policy include suggesting a cost-effective strategy to prevent vitamin D deficiency in infants by promoting collaboration at local, regional and national levels in Ireland.

3.7.3 Asia

Vitamin D deficiency is highly prevalent among the people residing in India even though sunlight exposure is available all year long. The urbanization of India, the darker pigmented skin, low use of supplements, and a lack of a national fortification of staple foods are several factors that lead to this insufficiency. An increased number of infants and children have bone deformities and rickets; however, the country has not prioritized vitamin D supplementation or fortification. Many inhabitants of India are vegetarian, limiting the dietary intake of vitamin D found in fish and meats. Due to a low use of supplements and a cultural diet that limits vitamin D intake, it is important to target food fortification with vitamin D as an alternative method.

One very common staple food among many Indians, vegetarians and non-vegetarians, is the whole-wheat 'chapatti' or bread. Fortifying the flour used to make this bread can help increase daily intake of vitamin D. Although in the United States and Canada milk is required to be vitamin D fortified, in India, many families, especially rural residents do not have access to fortified milk or facilities to store pasteurized milk (Babu and Calvo, 2010). Jordan is another country that was in a similar situation as India. Both countries receive sunlight all throughout the year. However,

in 2009, 87% Jordanians aged 18 to 70 had serum 25(OH)D levels less than 50 nmol/l (Babu and Calvo, 2010). Fortunately, the government of Jordan implemented a fortification policy for bread to help improve the vitamin D status. Urban populations had significantly lower serum 25(OH)D levels in comparison to rural populations (Babu and Calvo, 2010). It is important to note that there is no official recommendation for sufficient dietary intake for any age, gender, or factors such as pregnancy (Babu and Calvo, 2010). In order to provide national policies on supplementation, India first needs to implement a nationwide program that enables standard measurements of serum 25(OH)D. With these guidelines, pregnant women and infants can be targeted groups to prevent and treat vitamin D deficiency.

3.8 Barriers to supplementation

There are several challenges to incorporating vitamin D supplementation into public health policies. Each individual has a unique requirement for adequate vitamin D intake due to various exposure, genetics, and diet. The controversy of the recommended DRI is largely due to the gap in vitamin D research (Cashman and Kiely, 2011). However, recent advances in vitamin D knowledge enabled the IOM to propose estimated average requirements. More specifically, the evidence based proposal suggests a '40 nmol/l median value of a serum 25(OH)D level in which about 50% of the population might meet this requirement and a value of 50 nmol/l of a serum 25(OH)D level in which nearly all of the normal healthy population can meet this requirement (Cashman and Kiely, 2011).'

The IOM establishes the DRI values and the most recent review of vitamin D in 2010 utilized a risk assessment strategy. These steps include hazard identification, hazard characterization, intake assessment, and risk characterization. Once a report is completed, agencies can implement public health policies and educational programs to address the gaps identified in the risk assessment. One of the concerns for establishing guidelines for supplementation is the risk of consuming too little vitamin D levels resulting in no health benefits or consuming an increased level that would be toxic and result in adverse effects. Cashman and Kiely (2011) presents that utilizing the risk assessment approach as exemplified by the IOM has the potential to promote a national policy for vitamin D and improving international collaboration.

3.9 Conclusion

Vitamin D deficiency is a public health issue that has become pandemic to many regions of the world. As research advances on vitamin D and health, gaps are continually addressed and further research will help guide public health aims to eliminate the prevalence of vitamin D deficiency. A multi-strategy approach involving education, fortification, and supplementation is best to address the vitamin D problem. Education is key to informing the public of how to prevent vitamin D deficiency.

From a public health standpoint, fortifying foods common to a region or a whole population is a good way to increase vitamin D sources. Naturally occurring vitamin D in foods may not be consumed every day or there may be dietary cultural restrictions. Infants who exclusively breastfeed may need additional supplementation but most formulas are vitamin D fortified in the USA.

At an international level, once data on vitamin D deficiency prevalence in a community is quantified, health agencies or public health officials should recruit stakeholders to help obtain funding and disseminating knowledge about vitamin D insufficiency. A community-based approach in addressing this problem can be more sustainable in comparison to individual efforts. Economical and financial barriers can limit obtaining fortified or supplements to improve vitamin D deficiency in low and middle-income countries. With additional public health activities supported through funding, a larger population can be reached.

Public health programs that focus on supplementation can be either community based or clinical programs. Both need to incorporate cultural sensitivity, feasibility, and affordability. Individual compliance varies in attending clinics or taking supplements and therefore an educational component can increase support for supplementation through awareness. Overall, sustainability increases when people know the benefits of supplementation. Due to the toxicity concerns with vitamin D, it may be beneficial to channel supplementation programs through local clinics where supplementation can be based on blood tests. Recommended dosage levels and clinical advice can be personalized to an individual based upon medical history as well. In conclusion, supplementation requirements vary on an individual basis and therefore, individuals should seek the advice of a medical professional.

References

Babu, U. and Calvo, M., 2010. Modern India and the vitamin D dilemma: evidence for the need of a national food fortification program. Molecular Nutrition & Food Research 54, 1134-1147.

Calvo, M. and Whiting, S., 2006. Public health strategies to overcome barriers to optimal vitamin D status in populations with special needs. Journal of Nutrition 136, 1135-1139.

Cashman, K. and Kiely, M., 2011. Towards prevention of vitamin D deficiency and beyond: knowledge gaps and research needs in vitamin D nutrition and public health. British Journal of Nutrition 106, 1617-1627.

Centers for Disease Control and Prevention (CDC), 2008. Fat-soluble vitamins and micronutrients: vitamin D. Available at: http://www.cdc.gov/nutritionreport/part_2b.html.

De-Regil, L.M., Palacios, C., Ansary, A., Kulier, R. and Pena-Rosas, J.P., 2012. Vitamin D supplementation for women during pregnancy. Cochrane Database of Systematic Reviews 6. DOI: http://dx.doi.org/10.1002/14651858.CD008873.pub2.

Forrest, K. and Stuhldreher, W., 2011. Prevalence and correlates of vitamin D deficiency in US adults. Nutrition Research 31, 48-54.

Hathcock, J.N., Shao, A., Vieth, R. and Heaney, R., 2007. A risk assessment for vitamin D. American Journal of Clinical Nutrition 85, 6-18.

Health Service Executive (HSE), 2010. Policy on vitamin D supplementation for infants in Ireland. Available at: http://www.hse.ie/eng/services/healthpromotion/Vitamin_D_and_your_baby/Vitamin%20D%20Policy.pdf.

Holick, M. and Chen, T., 2008. Vitamin D deficiency: a worldwide problem with health consequences. American Journal of Clinical Nutrition 87, 1080S-1086S.

Institute of Medicine of the National Academies (IOM), 2010. DRIs for calcium and vitamin D. Available at: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/DRI-Values.aspx.

Laaksi, I.T., Ruohola, J.P., Ylikomi, T.J., Auvinen, A., Haataja, R.I., Pihlajamäki, H.K. and Tuohimaa, P.J., 2006. Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. European Journal of Clinical Nutrition 60, 1035-1038.

Lamberg-Allardt C., 2006. Vitamin D in foods and as supplements. Progress in Biophysics and Molecular Biology 92, 33-38.

Lips, P., 2007. Vitamin D status and nutrition in Europe and Asia. Journal of Steroid Biochemistry and Molecular Biology 103, 620-625.

Misra, M., Pacaud, D., Petryk, A., Collet-Solberg, P.F. and Kappy, M., 2008. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Paedatrics 122, 398-417.

Mohr, S., 2009. A brief history of vitamin D and cancer prevention. Annals of Epidemiology 19, 79-83.

Nakamura, K., Nashimoto, M., Hori, Y. and Yamamoto, M., 2000. Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. American Journal of Clinical Nutrition 71, 1161-1165.

National Institutes of Health: office of dietary supplements (ODS), 2011. Vitamin D. Available at: http://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/.

Office of Dietary Supplements (ODS), 2010. ODS vitamin D initiative. Available at: http://ods.od.nih.gov/research/VitaminD.aspx.

Public Advice International Foundation (PA International), 2012. Vitamin D nutrition policy in Europe. Available at: http://www.pa-international.org/Vitamin-D.htm.

Schwalfenberg, G. and Genuis, S., 2010. Vitamin D supplementation in a nursing home population. Molecular Nutrition and Food Research 54, 1072-1076.

Veith, R., 2007. Vitamin D toxicity, policy, and science. Journal Bone and Mineral Research 22, 64-68.

World Health Organization (WHO), 2011. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia. Available at: http://whqlibdoc.who.int/publications/2011/9789241548335_eng.pdf.

World Health Organization (WHO), 2012. Vitamin D supplementation to improve treatment outcomes among children diagnosed with respiratory infections. Available at: http://www.who.int/elena/titles/bbc/vitamind_pneumonia_children/en/index.html.

Zhu, Z., Zhan, J., Shao, J., Chen, W., Chen, L., Li, W., Ji, C. and Zhao, Z., 2012. High prevalence of vitamin D deficiency among children aged 1 month to 16 years in Hangzhou, China. BMC Public Health 12, 126.

Vitamin D and musculoskeletal health

Key facts

- Falls are the most common cause of accidental mortality and morbidity in older adults.
- Vitamin D insufficiency is common in older adults – among other adverse consequences this insufficiency can be associated with weakness and an increased risk of falls.

Summary points

- Among those at a high risk of falling (e.g. prior fall within the last year), a dose of 800-2,000 IU of vitamin D per day appears to be a safe economical intervention that can reduce their risk of future falls by up to 34%, particularly if they are deficient prior to treatment.
- Although not well understood, vitamin D most likely mediates its effect on falls through multiple pathways, including those involving skeletal muscle cells and neurotransmitter activity.
- The estimated effective serum 25-hydroxyvitamin D threshold to prevent falls is 60 nmol/l, to improve muscle strength and balance is 75 nmol/l, and possibly even higher to prevent fractures.

4. Vitamin D and fall risk

J. Basran[1], R.L. Duckham[2] and D.B. Hogan[3]
[1]Division of Geriatric Medicine, Department of Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B2, Canada; [2]College of Kinesiology, University of Saskatchewan, 87 Campus Drive, Saskatoon, SK S7N 5B2, Canada; [3]Division of Geriatric Medicine, Department of Medicine, Faculty of Medicine, University of Calgary, HSC-3330 Hospital Dr. NW, Calgary, Alberta T2N 4N1, Canada; jenny.basran@saskatoonhealthregion.ca

Abstract

Falls are a major health care concern, particularly in the growing older adult population. Falls not only contribute to escalating health care costs, but more importantly are associated with detriments to an individual's quality of life, as well as morbidity and mortality. The association between vitamin D deficiency and muscle weakness and/or worsening balance likely underlies the link between low serum 25-hydroxyvitamin D (25(OH)D) levels and increased rates of falls. Although the mechanisms by which vitamin D affects the likelihood of falling are not fully understood, adequate levels of this micronutrient enhance skeletal muscle strength and improve postural stability. Vitamin D3 at daily doses between 800-2,000 IU will elevate serum 25(OH)D levels to at least 60 nmol/l, a level (where a fall reduction effect has been demonstrated) in most older persons. Although still debated, we feel there is convincing evidence that vitamin D can reduce falls by between 13-34%. Vitamin D3 is a safe, economical, easily administered and well tolerated intervention that should be added to the customized fall prevention plan of high risk fallers.

Keywords: 25-hydroxyvitamin D, cholecalciferol, ergocalciferol, fall related injury, vitamin D receptors

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_4, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D, calcitriol
25(OH)D	25-hydroxyvitamin D
CI	Confidence interval
HR	Hazard ratio
IOM	US Institute of Medicine
OR	Odds ratio
RR	Relative risk
UVB	Ultraviolet B
VDR	Vitamin D receptor

4.1 Introduction

Falls are the most common cause of accidental injury and death among older adults (Kalyani *et al.*, 2010). While the definition of a fall seems self-evident, for clinical and research purposes a standardized definition is required to ensure accuracy and consistency. The Prevention of Falls Network, an international working group on falls, defines a fall as 'an unexpected event in which the participant comes to rest on the ground, floor or lower level' (Lamb *et al.*, 2005). Despite this definition, there remains uncertainty about whether to include events arising from a loss of consciousness or unusual external forces (e.g. strong push or shove) when counting falls.

As society ages, the number of fall-related hospitalizations is rapidly rising within the more economically developed nations of the world. In the United States, fall-related hospitalizations have increased from 373,128 to 559,355 between 2001 and 2008 (Hartholt *et al.*, 2011) while in the Netherlands, fall-related hospitalizations rose 137% between 1981 and 2008 among those 65 years of age and older (Hartholt *et al.*, 2010). Approximately one in three community dwelling adults aged sixty-five years and older (65+) experience at least one fall every year, a rate that increases to one in two adults over the age of 80 (Tinetti *et al.*, 1988). Between a quarter and a half of these older fallers will have multiple falls within that year (Souberbielle *et al.*, 2010), with up to 30% seeking medical attention as a result of a fall (Bischoff-Ferrari *et al.*, 2004; Peel, 2011).

In 2000, the estimated direct medical costs of falls among older adults in the US was over $19 billion dollars (or $25.6 billion dollars in 2012) (Stevens *et al.*, 2006). The most costly component (approximately $12 billion dollars) was for hospitalization costs, with expenditures for women 2-3 times higher than for men. Falls are the presenting complaint for between 18-40% of all emergency visits by older adults and account for over 80% of all injury related hospitalizations in this age group (Peel, 2011; Scott *et al.*, 2010). Fractures are both the most frequent and most expensive type of non-fatal injury, although head injuries are also a concern. Although thoracic spine and radius fractures frequently occur, falls account for 90% of all hip fractures (Zuckerman, 1996). The latter fracture is particularly concerning, given the high occurrence of

permanent disability and the one-year 20% mortality rate associated with fall related hip fractures (Zuckerman, 1996).

A recent Canadian report found that once hospitalized for a fall-related injury, an individual had an average acute care length of stay 70% longer than compared to the average length of stay for all other hospitalizations (Scott *et al.*, 2010). In addition, older adults in acute care facilities also have high rates of falls – up to 20 falls per 1000 bed-days (Haines and Waldron, 2011; Oliver *et al.*, 2004). Accidental inpatient falls are the most commonly reported safety incident (approximately 30%) with injuries occurring in almost of third (Oliver *et al.*, 2008; Peel, 2011).

The occurrence of falls is associated with an increased risk of institutionalization. Compared with similarly aged persons who have not fallen, those with a single non-injurious fall will have nearly a five-fold higher risk of admission to a long term care facility within three years. Among older persons with a fall causing serious injury the relative risk of admission is approximately twenty times higher (Tinetti and Williams, 1997). In an American study, the population attributable risk of a permanent admission to a long-term facility due to falls related causes was approximately 26% (Tinetti *et al.*, 1988). In other words, about a quarter of all permanent long term care admissions could be attributed to a fall. Not surprisingly falls are also frequent among the generally disabled group of seniors found in these facilities, where the incidence of falls is 1.5 falls per bed per year (Peel, 2011; Rubenstein *et al.*, 1994).

In addition to an injury, a fall may negatively impact an individual's quality of life by causing a fear of falling. This often triggers a vicious cycle where the fear initiates a restriction in activity leading to decreased mobility and social isolation causing a further increase in their risk of falling. The mortality, morbidity, disability, pain, other detrimental effects on health-related quality of life, and the growing economic burden of falls and related injuries underscore the need for effective interventions for fall prevention (Rosen, 2011a).

It has long been recognized that the more fall risk factors present, the greater the likelihood of falling (Tinetti *et al.*, 1988) and that most falls in older adults result from an inter-play of predisposing and precipitating factors (Berry and Miller, 2008). Predisposing factors include increasing age, female sex, age-associated changes in strength and balance, sensory impairments (e.g. poor vision, polyneuropathies, vestibulopathies), and chronic diseases (e.g. lower extremity arthritis and various neurological conditions like dementia, cerebrovascular disease, and extrapyramidal disorders). Falls are then precipitated in at risk individuals by such factors as: acute illnesses (e.g. delirium, sleep disturbances with fatigue, dehydration with orthostatic hypotension, or weakness), medications (e.g. psychotropics), lower urinary tract symptoms, orthostatic hypotension and weakness. This risk is further exacerbated by inappropriate footwear and environmental factors such as a wet floor and poor lighting, or being placed in a new and confusing environment (e.g. a hospital or a long term care facility). The current recommended approach to fall prevention focuses on searching for modifiable risk factors coupled with tailored interventions to deal with those factors identified. These interventions can include one or usually a combination of: patient education, adaptation or modification of home environment, withdrawal

or minimization of psychoactive and other medications, management of postural hypotension, management of foot problems and footwear, and exercise – particularly balance, strength, and gait training (Panel on Prevention of Falls in Older Persons and British Geriatrics, 2011).

Increasingly vitamin D supplementation has become recognized as an addition to the list of fall reduction interventions. It is particularly attractive due to its low cost and ease of implementation. In the rest of this chapter we will review the data linking vitamin D deficiency with fall risk and examine the evidence of benefit with supplementation.

4.2 Vitamin D and falls

The serum concentration of 25(OH)D is felt to be the 'best' single indicator of nutritional and functional status of vitamin D (Rosen, 2011b). Low levels of 25(OH)D encompasses both 'deficiency' (serum 25(OH)D levels <25 nmol/l) and 'insufficiency' (serum 25(OH)D levels 25-75 nmol/l) states (Hanley *et al.*, 2010). Risk factors for insufficiency or deficiency states include female sex, dark skin pigmentation, avoidance of ultraviolet radiation, diet low in food sources of vitamin D or malabsorption (though most circulating vitamin D is derived from sunlight exposure), obesity, and impaired renal function. Older adults are particularly prone to low levels of vitamin D and have diminished responsiveness of tissue targets of vitamin D due to reduced receptor numbers. Between the ages of 20 and 70 the ability to synthesize vitamin D in our skin declines by 75% (Holick, 2011). This is compounded by reduced exposure to sunlight in many older adults, particularly those in institutional care (Kalyani *et al.*, 2010; Menant *et al.*, 2012). With age, gastrointestinal absorption of vitamin D is also reduced, along with a decline in the metabolism to active forms of vitamin D by the kidneys and peripheral tissues (Muir and Montero-Odasso, 2011).

In the northern hemisphere, people who live above the 35° N latitude will have little or no vitamin D synthesis in their skin from November to February (Holick, 2004). Low levels of vitamin D are found in a third or more of adults in Canada and among those living in the northern half of the USA (Hanley and Davison, 2005; Liu *et al.*, 1997; Rucker *et al.*, 2002). Interestingly, low vitamin D levels are also common among those who fall and have been found to be an independent predictor of incident falls (Flicker *et al.*, 2003; Stein *et al.*, 1999). Nearly three-quarters of patients attending a falls clinic in the United Kingdom had a vitamin D deficiency (Dhesi *et al.*, 2002).

In addition to its effects on bone health, vitamin D has a variety of non-skeletal functions, including enhancing muscle health (Bischoff-Ferrari, 2012; Thacher and Clarke, 2011). Clinically, severe levels of vitamin D deficiency are associated with proximal muscle weakness, wasting, pain and changes in walking such as waddling or myopathic gait (the 'waddling' is due to the weakness of the proximal muscles of the pelvic girdle) (Glerup *et al.*, 2000; Stockton *et al.*, 2011). Benefits might arise from activating vitamin D receptors in muscle that in turn will promote protein synthesis. Observational studies have shown direct correlations between 25(OH)D levels and muscle strength as well as lower extremity function. Low levels of vitamin D are also specifically

associated with postural instability (Boersma *et al.*, 2012). Finally, numerous cross-sectional studies in community dwelling older adults have shown a direct relationship between vitamin D status and physical performance measures, especially when levels are low (Houston *et al.*, 2011; Muir and Montero-Odasso, 2011).

The foregoing all suggests that vitamin D supplementation might be an effective and feasible approach to fall prevention in older people (Annweiler *et al.*, 2010). Intervention studies have shown that supplemental vitamin D at daily doses of 800 to 1000 IU consistently lead to small but clinically significant improvements in surrogate markers for fall risk including improved lower extremity muscle strength, decreased postural sway, and lower 'Timed Up and Go' times but not for gait variables (Muir and Montero-Odasso, 2011; Zhu *et al.*, 2010). Most importantly, vitamin D supplementation has been associated with a 14 to 34% reduction in actual falls. The effect on rates varies depending on the dose of vitamin D used, how it was administered and the proportion with deficiency prior to the initiation of therapy (Annweiler *et al.*, 2010; Bischoff-Ferrari, 2012). With regards to the latter point, the effect appears to be more prominent in patients who are vitamin D deficient prior to the initiation of supplementation (Murad *et al.*, 2011).

4.3 Potential mechanisms

How vitamin D specifically reduces the likelihood of falls at a cellular level is still being elucidated but several theories exist. Activation of fast twitch (type II) muscle fibers occurs when an individual requires a fast reaction, such as would be needed in regaining balance to prevent a fall (Flicker *et al.*, 2003). Aging itself is associated with greater atrophy of these fast twitch (type II) muscle fibers relative to slow twitch fibers (Lips and Van Schoor, 2011). It is possible that the age related decline in fast twitch muscle fibers may, in part, be explained by vitamin D deficiency. Their loss could lead to an increased risk for falling (Bischoff-Ferrari *et al.*, 2004; Flicker *et al.*, 2003; Hamilton, 2010).

A number of studies (Bischoff-Ferrari *et al.*, 2004; Bischoff *et al.*, 2001; Costa *et al.*, 1986) suggested that the effect of vitamin D on muscle was likely direct and via genomic transcription (Bischoff-Ferrari, 2012). Earlier research showed VDRs to be present in all skeletal muscle cells. When VDRs are bound by active vitamin D metabolites (i.e. $1,25(OH)_2D$), protein synthesis is increased leading to muscle cell growth (Bischoff *et al.*, 2001; Freedman, 1999; Simpson *et al.*, 1985; Sorensen *et al.*, 1979). It has been argued that reduced VDR binding and expression eventually results in the sarcopenia seen at an advanced age (Bischoff-Ferrari *et al.*, 2004; Bischoff *et al.*, 2001; Simpson *et al.*, 1985). More recently, however, Wang and De Luca (2011) have rebutted the notion that VDRs are found in muscle. They suggest that earlier studies using chick monoclonal VDR antibody 9A7 identified non-VDR proteins, which stain with this antibody (Wang and DeLuca, 2011). They postulate that the effect of $1,25(OH)_2D$ on muscle function is likely indirect (Pfeifer *et al.*, 2001; Wang and DeLuca, 2011), possibly through the nervous and/or endocrine-metabolic systems (Binkley, 2012).

Vitamin D deficiency may alter neurotransmitter activity, thereby adversely affecting neurological function. VDR have been located at a cellular level in neuronal and glial cells (Binkley, 2012; Muir and Montero-Odasso, 2011; Vandervoort, 2002). With aging, VDR expression and activation will decline resulting in reductions in neural functions such as nerve conduction (Annweiler *et al.*, 2010; Dretakis *et al.*, 2010). This could result in poor balance and reduced reaction time (Ceglia, 2009; Gallagher *et al.*, 2007; Muir and Montero-Odasso, 2011). Moreover, impaired cognition from reduced VDR expression could lead to slower processing speeds in older adults (Kalueff and Tuohimaa, 2007; Menant *et al.*, 2012; Michael *et al.*, 2010).

An alternative mechanism that has been proposed is via changes in sex steroid levels. Consistent with the seasonal variation seen in vitamin D levels, there is distinct seasonal variability with testosterone. Highest levels are observed during the summer (Andersson *et al.*, 2003). As testosterone has anabolic effects on bone and muscle, it is possible that vitamin D supplementation in vitamin D deficient individuals might indirectly result from increases in circulating testosterone (Binkley, 2012). Further work to confirm or refute this suggested mechanism is required.

Which of these (or some other) mechanisms best explains the relationship between falls and vitamin D deficiency in older adults remains unknown (Janssen *et al.*, 2002). Given the breadth of literature, it is likely that vitamin D mediates its effect through multiple pathways.

4.4 Extent of fall risk reduction with vitamin D

In spite of the number of systematic reviews and meta-analyses, there continues to be controversy over the role of vitamin D for the specific indication of preventing falls. Over the last several years, a number of organizations have produced guidelines and/or position papers commenting on its utility in older individuals for this purpose (Table 4.1). While most other groups have endorsed the use of vitamin D for fall prevention in at least some circumstances, the IOM drew criticism and controversy by concluding that the evidence was too inconsistent to allow them to make a favorable recommendation (Ross *et al.*, 2011).

Recent systematic reviews and meta-analyses have indicated that vitamin D supplementation can lead to anywhere between a 13% to 34% reduction in falls, although there was moderate heterogeneity in the studies and potential publication bias (Table 4.2). The evidence for falls prevention was predominantly in patients who were vitamin D deficient and then received a dose greater than 700-800 IU once a day. Co-administering calcium was beneficial in subgroup analyses of some of the systematic reviews (Kalyani *et al.*, 2010; Murad *et al.*, 2011). While no difference in effect was seen when comparing those dwelling in the community with those institutionalized (Murad *et al.*, 2011) many of the community based studies recruited individuals at high risk for falls and/or deficient in vitamin D. Those enrolled may have had a closer resemblance to institutionalized adults than a more diverse community sample. Although fall rates are high in acute care, there is limited evidence for this setting. Despite a negative result, one acute care randomized controlled trial (RR of 800 IU of vitamin D on risk of falling = 0.82, 95% CI = 0.59-

Table 4.1. Guideline/ position paper summary.

Organization[1]	Recommendation
Endocrine Society 2011 (Holick *et al.*, 2011)	Recommends prescribing vitamin D supplementation for fall prevention.
IOF Position Paper 2010 (Dawson-Hughes *et al.*, 2010)	From a meta-analysis of available data, they concluded that a mean serum 25(OH)D level of at least 60 nmol/l (24 ng/ml) is needed for optimal fall risk reduction. Supplementation in amounts of 17.5 to 25 µg/day (700-1000 IU/day), lowered their risk of falling by 20% in older individuals, independent of their calcium intake level.
Osteoporosis Canada 2010 (Hanley *et al.*, 2010)	Evidence that supplementation with 20 µg (800 IU) vitamin D3 daily reduces the risk of falls, particularly from trials with adequate ascertainment of falls (level 2 evidence).
AGS/BGS Clinical practice guidelines – prevention of falls in older persons 2010 (Panel on prevention of falls in older persons and British geriatrics, 2011)	Vitamin D supplements of at least 800 IU per day should be considered for people with suspected vitamin D deficiency or who are otherwise at increased risk for falls [Strength of recommendation – B].
CADTH – Vitamin D supplementation in long term care residents: a review of the clinical effectiveness and guidelines (CADTH, 2010)	Overall, felt that the identified evidence supports vitamin D supplementation at a dose of at least 800 IU daily in residents of long-term care facilities to reduce the rate of falls.
ACSQHC – Prevention of falls and harm from falls in older adults (ACSQHC, 2009)	Vitamin D and calcium supplementation recommended as an intervention strategy to prevent falls in residents of residential aged care facilities (Level I).
The 2011 Report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine (Ross *et al.*, 2011)	Although future research may elucidate clear benefits and possibly even different requirement levels for vitamin D in relation to these non-skeletal outcomes (including falls), existing data cannot support such conclusions.

[1] ACSQHC: Australian Commission on Safety and Quality in Health; CADTH: Canadian Agency for Drugs and Technology in Health; IOF: International Osteoporosis Foundation.

1.16) (Burleigh *et al.*, 2007), did suggest a benefit trend and interest in the role of vitamin D supplementation for fall prevention in hospitalized seniors persists.

4.5 Type, dose and toxicity of vitamin D

The two main forms of vitamin D are D2 and D3. Vitamin D3 (cholecalciferol), is produced in the skin in response to UVB radiation. Lanolin can be used to produce vitamin D3 supplements and in some countries is used to fortify food (Holick, 2011). Vitamin D2 (ergocalciferol) is

Table 4.2. Summary of recent systematic reviews and meta-analysis.

Review	Conclusion
Murad *et al.*, 2011	Vitamin D use associated with 14% reduction in the risk of falls (OR for suffering at least one fall, 0.86; 95% CI = 0.77-0.96). This effect was more prominent in patients who were vitamin D deficient at baseline and in studies in which calcium was co-administered with vitamin D. The quality of evidence was low to moderate because of heterogeneity and publication bias. The majority of the evidence is derived from trials enrolling elderly women.
US Preventive Services Task Force – Systematic Review 2010 (Michael *et al.*, 2010)	Vitamin D with or without calcium associated with a 17% (95% CI = 11% to 23%) reduced risk for falling during 6 to 36 months of follow-up. Trials of vitamin D with calcium compared with no treatment or placebo did not support any added benefit of calcium. Age, sex distribution, history of falling, or risk status of the participants did not affect the pooled estimate.
Kalyani – JAGS – 2010 (Kalyani *et al.*, 2010)	In pooled analysis, vitamin D therapy (200-1000 IU) resulted in 14% (RR = 0.86, 95% CI = 0.79-0.93) fewer falls than calcium or placebo (number needed to treat =15).
BMJ 2009 (Bischoff-Ferrari *et al.*, 2009)	Pooled analysis of double blind randomized control trials, vitamin D therapy resulted in 13% lower risk of falls (RR = 0.87, 95% CI = 0.77-0.99), but high heterogeneity (Q= *P*=0.05). In analysis of trials with vitamin D dose equal or greater than 700 IU OD, the RR = 0.81 (CI = 0.71-0.92) and number needed to treat = 11 (95% CI = 7-20).
BMJ 2011 (Bischoff-Ferrari *et al.*, 2011)	Re-analysis by the same investigators indicated a 34% risk reduction for the higher (700-1000 IU/d) dose trials (OR = 0.66 (95% CI = 0.53-0.82)) and no reduction with lower doses of vitamin D (OR = 1.14 (95% CI = 0.69-1.87)), with a borderline significant interaction term (*P*=0.06).

made by yeast and mushrooms exposed to UVB radiation (Holick, 2011). It is often given as high dose boluses when used for treatment. Both forms of vitamin D can be metabolized to 1,25-dihydroxyvitamin D, the active form of the vitamin. The two forms of vitamin D appear to be effective in falls reduction, although vitamin D3 has been more commonly used in studies as there is some evidence that D2 may not be used as efficiently in *in vivo* studies (Armas *et al.*, 2004; Kalyani *et al.*, 2010; Murad *et al.*, 2011). There is less evidence available on other forms of vitamin D as it relates to fall prevention. No significant reduction in falls was found with alfacalcidol (RR = 0.84, 95% CI = 0.58-1.22) (Kalyani *et al.*, 2010). A few trials have been performed using the 25(OH)D3 metabolite itself. It is more hydrophilic than the parent compound, has a short half life (8 to 11 hours), and causes a rapid increase in serum 25(OH)D levels. Although, trials have shown improvements in bone density, the mechanical strength of bone, and lower extremity function, an effect on fall rates has not been reported (Bischoff-Ferrari *et al.*, 2012).

Adherence is not a major issue with oral vitamin D supplement. Missing a dose now and then when taken on a daily basis would not be expected to lead to significant issues. However there remains interest in looking at different dosing approaches to quickly and reliably reach desired levels of 25(OH)D. The estimated effective threshold of 25(OH)D is 60 nmol/l to prevent falls, 75 nmol/l to improve muscle strength and balance, and possibly even higher for fracture prevention (Dawson-Hughes, 2012; Glendenning and Inderjeeth, 2012; Muir and Montero-Odasso, 2011). Relatively long periods of time, at least three months, are required with regular oral supplementation with vitamin D3 to achieve these levels (Bischoff-Ferrari *et al.*, 2012; Hanley *et al.*, 2010). Hanley provides a clinically useful estimate, whereby 1 μg (40 IU) of vitamin D3 will increase serum 25(OH)D by 0.7-2.0 nmol/l (Hanley *et al.*, 2010). For most adults, a supplement of 20-25 μg (800-1000 IU) daily will raise serum 25(OH)D levels by approximately 15-30 nmol/l in about 3 months. It is unclear, though, whether getting to these desired levels any quicker is important. 25(OH)D3 metabolite at a dose of 20 μg, reached a 25(OH)D threshold greater than 75 nmol/l in about a month, but has not yet been shown to be effective in fall prevention (Bischoff-Ferrari *et al.*, 2012).

Three trials have raised concerns that high-dose bolus therapy may have overall detrimental effects on health. Smith *et al.* (2007) administered 300,000 IU ergocalciferol (vitamin D2) intramuscularly annually for three years. There was no effect on falls (HR = 0.98 (0.93-1.04), but there was a significant increase in the risk of hip, femur and wrist fractures among women in the treatment group (HR = 1.59; 95% CI = 1.17-2.16, *P*=0.003) (Smith *et al.*, 2007). Sanders *et al.* (2010), administered 500,000 IU of oral cholecalciferol annually for 3 years and found a 26% increased risk of fractures (RR = 1.26; 95% CI = 1.00-1.59, *P*=0.047) and a 15% increased risk of falls (RR = 1.15; 95% CI = 1.02-1.30, *P*=0.03) with treatment compared to the control arm (Sanders *et al.*, 2010). Recently, Glendenning *et al.* (2012) administered 150,000 IU oral cholecalciferol every 3 months for a total of 9 months. Although there were no statistically significant differences in either the number of falls or the measures of mobility or hand strength compared to placebo, there was a trend suggesting the treatment group was more likely to experience multiple falls as compared to the control group (OR = 1.58; CI = 0.83-2.99) (Glendenning *et al.*, 2012).

Why would bolus therapy be detrimental? Rosen (2011a) suggested the following explanations: (1) high doses generate higher concentrations of dangerous metabolites; (2) vitamin D, a prohormone, acts in a catabolic manner at high doses (like parathyroid hormone); or (3) that high serum levels are dangerous, a reverse J-curve, as suggested by the IOM report (while levels between 50-80 nmol/l are good, both low levels and levels >80 nmol/l are associated with health issues) (Rosen, 2011a).

Though serum 25(OH)D levels above 80-125 nmol/l are potentially harmful, toxicity is rare and occurs when vitamin D doses exceed 10,000 IU per day, with serum 25(OH)levels >375 nmol/l (Jones, 2008). Toxicity, if it occurs, typically presents as hypercalecemia, possibly nephrolithiasis, and/or renal damage. Caution should be used in conditions that can be associated with hypercalcemia and/or hypercalciuria such as the chronic granulomatous disorder sarcoidosis. With a half life of 15-20 days (Hanley *et al.*, 2010), vitamin D can accumulate in the body and take

a long time to clear so any toxicity may have long lasting effects. In Canada, daily intakes of up to 2,000 IU are considered safe without the need for any monitoring of vitamin D levels (Hanley *et al.*, 2010). The IOM reported the risk for harm begins to increase (upper tolerable level) at dosages beyond 4,000 IU/d and serum 25(OH)D levels >125 nmol/l (Ross *et al.*, 2011).

There is no need to obtain 25(OH)D levels either before or during therapy (Hanley *et al.*, 2010). Monitoring should be reserved for those with recurrent osteoporotic fractures, continued bone loss in spite of treatment, or co-morbid conditions that might affect vitamin D absorption or action (e.g. malabsorptive states, anticonvulsant therapy). The increase in the volume of questionably useful vitamin D testing has been staggering (Sattar *et al.*, 2012). In the Canadian province of Ontario the number of tests increased over 20-fold in only five years from 29,000 (2004) to over 700,000 (2009) (CBC News: http://www.cbc.ca/news/canada/calgary/story/2011/01/28/calgary-vitamin-d-blood-testing.html). Aside from issues of cost (the cost per test in Canada is $50 to $100 CDN) and lack of demonstrated benefit from the expenditure, there is significant inter-assay and inter-laboratory variability in the 25(OH)D assay (Lai *et al.*, 2010).

Researchers and clinicians alike have shown increasing interest in vitamin D as demonstrated by the sharp rise in the number of vitamin D publications. The lack of agreement on the benefit of vitamin D supplementation in reducing fall risk could be partially explained by the noted hetereogenity in the published studies and methodological shortcomings. Most community-based studies selectively recruited patients at a high risk of falling. By potentially exaggerating the risk reduction seen, generalizability of the results obtained is limited. Research in the area of fall prevention with vitamin D would benefit from more inclusive recruitment of subjects, careful assessment of potential confounders and explanatory variables (e.g. 25(OH)D levels), use of an array of outcome measures (including carefully ascertained falls and objective measures of strength, balance, and gait) and studies of a longer duration.

4.6 Conclusions

We feel there is compelling evidence that low vitamin D levels are associated with muscle weakness and impaired balance. This likely underlies the documented higher fall rates found in vitamin D deficient individuals. Although there are inconsistencies in the evidence base, the dominant theme is that vitamin D supplementation among those with low pre-treatment levels can reduce of further falls by as much as a third. To achieve benefits in fall prevention a 25(OH)D level of at least 60-75 nmol/l is required.

Pending the results of further research, what can be suggested now? Current recommendations for vitamin D intake in older adults have increased to 800 IU or more per day. Among those at a high risk of falling (e.g. prior fall within the last year), we would routinely suggest a dose of 800-2,000 IU per day. This dose range appears to be safe. It can be given with or without calcium (we normally recommend calcium supplementation). This dose range of vitamin D should guarantee vitamin D sufficiency unless there are mitigating circumstances. In addition, we encourage

exposure to natural sunlight in moderation (e.g. 5-15 minutes, 3 times a week between 10:00 and 15:00 during the summer) (Holick, 2011). At this time, intermittent high-dose therapy with vitamin D is not recommended.

Moderate vitamin D supplementation is an easy and inexpensive, yet effective, addition to any care plan for the reduction of falls and fall related injuries in older adults.

References

Andersson, A.M., Carlsen, E., Petersen, J. and Skakkeback, N., 2003. Variation in levels of serum inhibin B, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and sex hormone-binding globulin in monthly samples from healthy men during a 17-monthperiod: possible effects of seasons. Journal Clinical Endocrinology Metabolism 88, 932-937.

Annweiler, C., Montero-Odasso, M., Schott, A.M., Berrut, G., Fantino, B. and Beauchet, O., 2010. Fall prevention and vitamin D in the elderly: an overview of the key role of the non-bone effects. Journal of Neuroengineering & Rehabilitation 7, 50.

Armas, L.A.G., Hollis, B.W. and Heaney, R.P., 2004. Vitamin D(2) is much less effective than vitamin D(3) in humans. Journal of Clinical Endocrinology & Metabolism 89, 5387-5391.

Australian Commission on Safety and Quality in Health Care, 2009. Prevention of falls and harm from falls in older adults.

Berry, S.D. and Miller, R.R., 2008. Falls: epidemiology, pathophysiology, and relationship to fracture. Current Osteoporosis Reports 6, 149-154.

Binkley, N., 2012. Vitamin D and osteoporosis-related fracture. Archives of Biochemistry and Biophysics.

Bischoff-Ferrari, H., 2012. Relevance of vitamin D in muscle health. Reviews in Endocrine & Metabolic Disorders 13, 71-77.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Staehelin, H.B., Orav, J.E., Stuck, A.E., Theiler, R., Wong, J.B., Egli, A., Kiel, D.P. and Henschkowski, J., 2009. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ 339, b3692.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Stöcklin, E., Sidelnikov, E., Willett, W.C., Edel, J.O., Stähelin, H.B., Wolfram, S., Jetter, A., Schwager, J., Henschkowski, J., von Eckardstein, A. and Egli, A., 2012. Oral supplementation with 25(OH)D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. Journal of Bone and Mineral Research 27, 160-169.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Willett, W.C., Staehelin, H.B., Bazemore, M.G., Zee, R.Y. and Wong, J.B., 2004. Effect of vitamin D on falls: a meta-analysis. JAMA 291, 1999-2006.

Bischoff-Ferrari, H.A., Willett, W.C., Orav, J.E., Kiel, D.P. and Dawson-Hughes, B., 2011. Authors' reply. BMJ 342.

Bischoff, H.A., Borchers, M., Gudat, F., Duermueller, U., Theiler, R., Stahelin, H.B. and Dick, W., 2001. In situ detection of 1,25-dihydroxyvitamin D-3 receptor in human skeletal muscle tissue. Histochemical Journal 33, 19-24.

Boersma, D., Demontiero, O., Mohtasham Amiri, Z., Hassan, S., Suarez, H., Geisinger, D., Suriyaarachchi, P., Sharma, A. and Duque, G., 2012. Vitamin d status in relation to postural stability in the elderly. Journal of Nutrition, Health & Aging 16, 270-275.

Burleigh, E., McColl, J. and Potter, J., 2007. Does vitamin D stop inpatients falling? A randomised controlled trial. Age & Ageing 36, 507-513.

Canadian Agency for Drugs and Technology in Health, 2010. Vitamin D supplementation in long term care residents. A review of the clinical effectiveness and guidelines. Available at: http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.194.1683.

Ceglia, L., 2009. Vitamin D and its role in skeletal muscle. Current Opinion in Clinical Nutrition & Metabolic Care 12, 628-633.

Costa, E.M., Blau, H.M. and Feldman, D., 1986. 1,25-dihydroxyvitamin-D3 receptors and hormonal responses in cloned human skeletal muscle cells. Endocrinology 119, 2214-2220.

Dawson-Hughes, B., 2012. Serum 25-hydroxyvitamin D and muscle atrophy in the elderly. Proceedings of the Nutrition Society 71, 46-49.

Dawson-Hughes, B., Mithal, A., Bonjour, J.P., Boonen, S., Burckhardt, P., Fuleihan, G.E.H., Josse, R.G., Lips, P., Morales-Torres, J. and Yoshimura, N., 2010. IOF position statement: vitamin D recommendations for older adults. Osteoporosis International 21, 1151-1154.

Dhesi, J.K., Moniz, C., Close, J.C.T., Jackson, S.H.D. and Allain, T.J., 2002. A rationale for vitamin D prescribing in a falls clinic population. Age & Ageing 31, 267-271.

Dretakis, O.E., Tsatsanis, C., Fyrgadis, A., Drakopoulos, C.G., Steriopoulos, K. and Margioris, A.N., 2010. Correlation between serum 25-hydroxyvitamin D levels and quadriceps muscle strength in elderly cretans. Journal of International Medical Research 38, 1824-1834.

Flicker, L., Mead, K., MacInnis, R.J., Nowson, C., Scherer, S., Stein, M.S., Thomasx, J., Hopper, J.L. and Wark, J.D., 2003. Serum vitamin D and falls in older women in residential care in Australia. Journal of the American Geriatrics Society 51, 1533-1538.

Freedman, L.P., 1999. Transcriptional targets of the vitamin D-3 receptor-mediating cell cycle arrest and differentiation. Journal of Nutrition 129, 581S-586S.

Gallagher, J.C., Rapuri, P.B. and Smith, L.M., 2007. An age-related decrease in creatinine clearance is associated with an increase in number of falls in untreated women but not in women receiving calcitriol treatment. Journal of Clinical Endocrinology & Metabolism 92, 51-58.

Glendenning, P. and Inderjeeth, C.A., 2012. Screening for vitamin D deficiency: defining vitamin D deficiency, target thresholds of treatment and estimating the benefits of treatment. Pathology 44, 160-165.

Glendenning, P., Zhu, K., Inderjeeth, C., Howat, P., Lewis, J.R. and Prince, R.L., 2012. Effects of three-monthly oral 150,000 IU cholecalciferol supplementation on falls, mobility, and muscle strength in older postmenopausal women: A randomized controlled trial. Journal of Bone and Mineral Research 27, 170-176.

Glerup, H., Mikkelsen, K., Poulsen, L., Hass, E., Overbeck, S., Andersen, H., Charles, P. and Eriksen, E.F., 2000. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. Calcified Tissue International 66, 419-424.

Haines, T.P. and Waldron, N.G., 2011. Translation of falls prevention knowledge into action in hospitals: what should be translated and how should it be done? Journal of Safety Research 42, 431-442.

Hamilton, B., 2010. Vitamin D and human skeletal muscle. Scandinavian Journal of Medicine & Science in Sports 20, 182-190.

Hanley, D.A., Cranney, A., Jones, G., Whiting, S.J. and Leslie, W.D., 2010. Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada (summary). Canadian Medical Association Journal 182, 1315.

Hanley, D.A. and Davison, K.S., 2005. Vitamin D insufficiency in North America. Journal of Nutrition 135, 332-337.

Hartholt, K., Stevens, J., S, P., Van der Cammen, T. and Patka, P., 2011. Increase in fall-related hospitalizations in the United States, 2001-2008. Journal of Trauma 71, 255-258.

Hartholt, K.A., van der Velde, N., Looman, C.W.N., van Lieshout, E.M.M., Panneman, M.J.M., van Beeck, E.F., Patka, P. and van der Cammen, T.J.M., 2010. Trends in fall-related hospital admissions in older persons in the Netherlands. Archives of Internal Medicine 170, 905-911.

Holick, M.F., 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. American Journal of Clinical Nutrition 80, 1678S-1688S.

Holick, M.F., 2011. Vitamin D: a d-lightful solution for health. Journal of Investigative Medicine 59, 872-880.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M., 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. Journal of Clinical Endocrinology & Metabolism 96, 1911-1930.

Houston, D.K., Tooze, J.A., Davis, C.C., Chaves, P.H.M., Hirsch, C.H., Robbins, J.A., Arnold, A.M., Newman, A.B. and Kritchevsky, S.B., 2011. Serum 25-hydroxyvitamin D and physical function in older adults: the cardiovascular health study all stars. Journal of the American Geriatrics Society 59, 1793-1801.

Janssen, H.C.J.P., Samson, M.M. and Verhaar, H.J.J., 2002. Vitamin D deficiency, muscle function, and falls in elderly people. American Journal of Clinical Nutrition 75, 611-615.

Jones, G., 2008. Pharmacokinetics of vitamin D toxicity. American Journal of Clinical Nutrition 88, 582S-586S.

Kalueff, A.V. and Tuohimaa, P., 2007. Neurosteroid hormone vitamin D and its utility in clinical nutrition. Current Opinion in Clinical Nutrition and Metabolic Care 10, 12-19.

Kalyani, R.R., Stein, B., Valiyil, R., Manno, R., Maynard, J.W. and Crews, D.C., 2010. Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis. Journal of the American Geriatrics Society 58, 1299-1310.

Lai, J.K.C., Lucas, R.M., Clements, M.S., Harrison, S.L. and Banks, E., 2010. Assessing vitamin D status: pitfalls for the unwary. Molecular Nutrition & Food Research 54, 1062-1071.

Lamb, S.E., Jorstad-Stein, E.C., Hauer, K., Becker, C. and Prevention Falls Network, E., 2005. Development of a common outcome data set for fall injury prevention trials: The prevention of falls network Europe consensus. Journal of the American Geriatrics Society 53, 1618-1622.

Lips, P. and Van Schoor, N.M., 2011. The effect of vitamin D on bone and osteoporosis. Best Practice & Research Clinical Endocrinology & Metabolism 25, 585-591.

Liu, B.A., Gordon, M., Labranche, J.M., Murray, T.M., Vieth, R. and Shear, N.H., 1997. Seasonal prevalence of vitamin D deficiency in institutionalized older adults. Journal of the American Geriatrics Society 45, 598-603.

Menant, J., Close, J., Delbaere, K., Sturnieks, D., Trollor, J., Sachdev, P., Brodaty, H. and Lord, S., 2012. Relationships between serum vitamin D levels, neuromuscular and neuropsychological function and falls in older men and women. Osteoporosis International 23, 981-989.

Michael, Y.L., Whitlock, E.P., Lin, J.S., Fu, R., O'Connor, E.A., Gold, R. and Force, U.S.P.S.T., 2010. Primary care-relevant interventions to prevent falling in older adults: a systematic evidence review for the US preventive services task force. Annals of Internal Medicine 153, 815-825.

Muir, S.W. and Montero-Odasso, M., 2011. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. Journal of the American Geriatrics Society 59, 2291-2300.

Murad, M.H., Elamin, K.B., Abu Elnour, N.O., Elamin, M.B., Alkatib, A.A., Fatourechi, M.M., Almandoz, J.P., Mullan, R.J., Lane, M.A., Liu, H., Erwin, P.J., Hensrud, D.D. and Montori, V.M., 2011. Clinical review: The effect of vitamin D on falls: a systematic review and meta-analysis. Journal of Clinical Endocrinology & Metabolism 96, 2997-3006.

Oliver, D., Daly, F., Martin, F.C. and McMurdo, M.E.T., 2004. Risk factors and risk assessment tools for falls in hospital in-patients: a systematic review. Age and Ageing 33, 122-130.

Oliver, D., Killick, S., Even, T. and Willmott, M., 2008. Do falls and falls-injuries in hospital indicate negligent care – and how big is the risk? A retrospective analysis of the NHS Litigation Authority Database of clinical negligence claims, resulting from falls in hospitals in England 1995 to 2006. Quality and Safety in Health Care 17, 431-436.

Panel on Prevention of Falls in Older Persons, A.G.S. and British Geriatrics, S., 2011. Summary of the updated American geriatrics society/British geriatrics society clinical practice guideline for prevention of falls in older persons. Journal of the American Geriatrics Society 59, 148-157.

Peel, N.M., 2011. Epidemiology of falls in older age. Canadian Journal on Aging 30, 7-19.

Pfeifer, M., Begerow, B., Minne, H.W., Schlotthauer, T., Pospeschill, M., Scholz, M., Lazarescu, A.D. and Pollahne, W., 2001. Vitamin D status, trunk muscle strength, body sway, falls, and fractures among 237 postmenopausal women with osteoporosis. Experimental & Clinical Endocrinology & Diabetes 109, 87-92.

Rosen, C.J., 2011a. Nutrition: Vitamin D and falls[mdash]are intermittent, high doses better? Nature Reviews Endocrinology 7, 695-696.

Rosen, C.J., 2011b. Vitamin D insufficiency. New England Journal of Medicine 364, 248-254.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 Report on Dietary Reference Intakes for calcium and vitamin D from the institute of medicine: what clinicians need to know. Journal of Clinical Endocrinology & Metabolism 96, 53-58.

Rubenstein, L.Z., Josephson, K.R. and Robbins, A.S., 1994. Falls in the nursing-home. Annals of Internal Medicine 121, 442-451.

Rucker, D., Allan, J.A., Fick, G.H. and Hanley, D.A., 2002. Vitamin D insufficiency in a population of healthy western Canadians. Canadian Medical Association Journal 166, 1517-1524.

Sanders, K.M., Stuart, A.L., Williamson, E.J., Simpson, J.A., Kotowicz, M.A., Young, D. and Nicholson, G.C., 2010. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 303, 1815-1822. Erratum in JAMA 303, 2357.

Sattar, N., Welsh, P., Panarelli, M. and Forouhi, N., 2012. Increasing requests for vitamin D measurements: costly, confusing, and without credibility. Lancet 379, 95-96.

Scott, V., Wagar, L. and Elliott, S., 2010. Falls & related injuries among older canadians: fall related hospitalizations & intervention initiatives. Division of Aging and Seniors, Public Health Agency of Canada, Victoria Scott Consulting, Victoria, BC, Canada.

Simpson, R.U., Thomas, G.A. and Arnold, A.J., 1985. Identification of 1,25-dihyroxyvitamin-D3 receptors and activities in muscle. Journal of Biological Chemistry 260, 8882-8891.

Smith, H., Anderson, F., Raphael, H., Maslin, P., Crozier, S. and Cooper, C., 2007. Effect of annual intramuscular vitamin D on fracture risk in elderly men and women – a population-based, randomized, double-blind, placebo-controlled trial. Rheumatology 46, 1852-1857.

Sorensen, O.H., Lund, B.I., Saltin, B., Lund, B.J., Andersen, R.B., Hjorth, L., Melsen, F. and Mosekilde, L., 1979. Myopathy in bone loss of aging – improvement by treatment with 1alpha-hydroxycholecalciferol and calcium. Clinical Science 56, 157-161.

Souberbielle, J.-C., Body, J.-J., Lappe, J.M., Plebani, M., Shoenfeld, Y., Wang, T.J., Bischoff-Ferrari, H.A., Cavalier, E., Ebeling, P.R., Fardellone, P., Gandini, S., Gruson, D., Guerin, A.P., Heickendorff, L., Hollis, B.W., Ish-Shalom, S., Jean, G., von Landenberg, P., Largura, A., Olsson, T., Pierrot-Deseilligny, C., Pilz, S., Tincani, A., Valcour, A. and Zittermann, A., 2010. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. Autoimmunity Reviews 9, 709-715.

Stein, M.S., Wark, J.D., Scherer, S.C., Walton, S.L., Chick, P., Di Carlantonio, M., Zajac, J.D. and Flicker, L., 1999. Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel. Journal of the American Geriatrics Society 47, 1195-1201.

Stevens, J.A., Corso, P.S., Finkelstein, E.A. and Miller, T.R., 2006. The costs of fatal and non-fatal falls among older adults. Injury Prevention 12, 290-295.

Stockton, K.A., Mengersen, K., Paratz, J.D., Kandiah, D. and Bennell, K.L., 2011. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. Osteoporosis International 22, 859-871.

Thacher, T.D. and Clarke, B.L., 2011. Vitamin D insufficiency. Mayo Clinic Proceedings 86, 50-60.

Tinetti, M.E., Speechley, M. and Ginter, S.F., 1988. Risk factors for falls among elderly persons living in the community. New England Journal of Medicine 319, 1701-1707.

Tinetti, M.E. and Williams, C.S., 1997. Falls, injuries due to falls, and the risk of admission to a nursing home. New England Journal of Medicine 337, 1279-1284.

Vandervoort, A.A., 2002. Aging of the human neuromuscular system. Muscle & Nerve 25, 17-25.

Wang, Y. and DeLuca, H.F., 2011. Is the vitamin D receptor found in muscle? Endocrinology 152, 354-363.

Zhu, K., Austin, N., Devine, A., Bruce, D. and Prince, R.L., 2010. A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. Journal of the American Geriatrics Society 58, 2063-2068.

Zuckerman, J.D., 1996. Hip fracture. New England Journal of Medicine 334, 1519-1525.

Key facts

- The primary function of vitamin D is the maintenance of calcium and phosphate homeostasis and skeletal integrity throughout life.
- Vitamin D deficiency and/or insufficiency are global problems for growing children and adolescents.
- Vitamin D deficiency might arise from an inadequate supply, impaired utilization or enhanced excretion of vitamin D.
- An adequate vitamin D status is associated with higher muscle strength and muscle functions in children and adolescents.

Summary points

- High proportions of vitamin D deficiency and insufficiency have been reported in apparently healthy children and adolescents worldwide.
- A persistent severe vitamin D deficiency results in the bone disorder disease of rickets in children and osteomalacia in adults.
- Vitamin D deficiency is associated with an increase in parathyroid hormone concentration in blood, increase rate of bone turnover and a decreased rate of bone mass accumulation in growing children and adolescents.
- An inadequate vitamin D status exerts detrimental effect on musculoskeletal health in growing populations.
- Preventing vitamin D deficiency by ensuring an adequate vitamin D supply throughout childhood and adolescence might enhance physical growth and bone mass accretion, which ultimately could reduce the risk of osteoporotic fracture later in life.

5. The beneficial effects of higher (adequate) vitamin D status on bone growth and muscle strength in children and adolescents

L.H. Foo[1] and S.J. Whiting[2]
[1]Program of Nutrition, School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Kelantan, Malaysia; [2]Division of Nutrition and Dietetics, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatchewan, SK S7N 5C9, Canada; lhfoo@kb.usm.my

Abstract

Vitamin D is chemically a seco-steroid, which acts biologically as a prohormone and requires two hydroxylation processes, first in liver and the other in kidney (for those functions related to bone) before becoming active. The primary function of vitamin D is thought to be the maintenance of calcium and phosphate homeostasis and skeletal integrity throughout life. Numerous epidemiological studies suggest that vitamin D deficiency and/or insufficiency are global problems for growing children and adolescents. Although it is well established that persistent severe vitamin D deficiency results in the development of rickets in children and osteomalacia in adults, it is now suggested that a low 25-hydroxyvitamin D concentration is not only a biochemical abnormality, but also associated with physiological, pathological and clinical signs of vitamin D deficiency, along with secondary hyperparathyroidism, and increased bone remodelling across the lifespan. A growing body of evidence indicates that higher and adequate vitamin D status in the body plays an important role in maintaining musculoskeletal integrity at any age. However, the adverse effects of vitamin D deficiency on bone mass has been studied extensively in the postmenopausal women and in the elderly with only a few studies in children and adolescents. Therefore, the review will discuss the current evidence pertaining to the relationships between vitamin D status and musculoskeletal health in children and adolescents.

Keywords: vitamin D deficiency, musculoskeletal health, bone growth, children, adolescents

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_5, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
aBMD	Areal bone mineral density
BMC	Bone mineral content
BMD	Bone mineral density
DRI	Dietary reference intake
DXA	Dual-photon energy X-ray absorptiometry
PTH	Parathyroid hormone
RDA	Recommended dietary allowance
Vitamin D2	Ergocalciferol
Vitamin D3	Cholecalciferol

5.1 Introduction

Osteoporosis is defined as a systemic skeletal disorder characterised by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture risk (World Health Organization, 1994). Osteoporotic fractures result in substantial mortality, morbidity and are destructive in terms of productivity and quality of life. The economic and social burden of such fractures will increase dramatically worldwide as the population ages. So serious are the long-term consequences of osteoporotic fracture, in terms of economic and human cost that osteoporosis has been recognized as a major public health concern and a major contributor to medical care costs in many regions of the world (Cummings and Melton, 2002; Johnell and Kanis, 2004). A growing body of evidence suggested that osteoporosis may have its origins at an earlier stage in life by failure to achieve optimal peak bone mass during childhood and adolescence. Peak bone mass is defined as the amount of bony tissue present at the end of skeletal maturation (Heaney *et al.*, 2000). The quantity of bone when peak bone mass is reached is regarded as a major determinant of the risk of osteoporotic fractures later in life. Indeed, the adolescent years determine lifelong skeletal health because this period represents the time of greatest skeletal growth, during which bone mass is largely accrued. For instance, almost 40% of the bone mass accrual in girls occurs during this period (Bailey *et al.*, 1999; Matkovic *et al.*, 1994). Hence, osteoporotic fractures related to bone loss in later life may perhaps be prevented by strategies to promote and maximize bone mineralization during growing years. Therefore, better understanding the factors associated with greater bone mass accretion in growing children and adolescents is ultimately crucial, in order to prevent excessive age-related bone loss and osteoporotic fractures later in life. Of all these factors, vitamin D nutrition has been emerging as an important factor in relation to musculoskeletal health across the lifespan.

It is generally agreed that persistent severe vitamin D deficiency results in the development of rickets in children and osteomalacia in adults. On the other hand, mild vitamin D deficiency as known as hypovitaminosis D is regarded as an emerging global health problem for growing children and adolescents, with the prevalence of vitamin D deficiency increasingly being

reported in apparently healthy children and adolescents worldwide, in both the developed and the developing countries (Arabi *et al.*, 2010; Docio *et al.*, 1998; Foo *et al.*, 2009a; Fraser, 2004; Gordon *et al.*, 2004; Guillemant *et al.*, 1999; Lehtonen-Veromaa *et al.*, 2002a; Looker *et al.*, 2002). Surprisingly, hypovitaminosis D has been found in sunny countries at tropical latitudes where cutaneous production of vitamin D can occur throughout the year (Arabi *et al.*, 2010; El-Hajj Fuleihan *et al.*, 2001; Gannage-Yared *et al.*, 2000; Hatun *et al.*, 2005). Long term vitamin D deficiency is associated with increased parathyroid hormone levels in blood. Persistent elevation of PTH results in a net loss of bone, and in children, the development of rickets. In contrast, little is known about the effects on musculoskeletal health in children and adolescents of low vitamin D status without clinical signs of rickets. Nevertheless, several studies have found that vitamin D deficiency and insufficiency in children and adolescents resulted in secondary increases in serum PTH (El-Hajj Fuleihan *et al.*, 2001; Foo *et al.*, 2009a; Gordon *et al.*, 2004; Guillemant *et al.*, 1999; Outila *et al.*, 2001), increased bone turnover (Cheng *et al.*, 2003; Foo *et al.*, 2009b; Lehtonen-Veromaa *et al.*, 2002b;) and a decrease in the rate of bone mass accumulation (Foo *et al.*, 2009b; Jones and Dwyer, 1998; Lehtonen-Veromaa *et al.*, 2002b).

5.2 Effects of vitamin D status on bone growth in children and adolescents

A growing body of evidence which suggest that mild vitamin D deficiency also called 'insufficiency' in children and adolescents may also be deleterious to bone health in children and adolescents. Adequate vitamin D status may be important for skeletal health because of its primary function in maintaining calcium and phosphate homeostasis (Fraser, 1995). Numerous studies performed in growing children and adolescents have found that a persistently low vitamin D status with prolonged elevation of PTH and a subsequent increase in bone turnover, which consequently leads to sub-optimal bone mass accretion. However, the positive effects of vitamin D status on bone mass accretion in healthy growing children and adolescents have produced inconsistent results, especially among adolescents at the time of pubertal growth development. In Caucasian adolescent girls, a positive association has been found in some studies between vitamin D status and bone mass measurements (Cheng *et al.*, 2003; Lehtonen-Veromaa *et al.*, 2002b; Outila *et al.*, 2001). However, this association was not found in other studies (Kristinsson *et al.*, 1998; Willett, 2005). In a cross sectional survey of adolescent girls aged 14 to 16 years, a positive association was found between serum 25(OH)D concentrations and forearm aBMD, but only in adolescents who had a 25(OH)D levels of <40 nmol/l (Outila *et al.*, 2001). Similarly, in a 3-year longitudinal investigation of bone development in Finnish adolescent girls aged 9 to 15 years, Lehtonen-Veromaa *et al.*, (2002b), found a positive association between changes of serum 25(OH)D levels from baseline as well as the baseline 25(OH)D levels themselves on lumbar spine aBMD. Both studies used aBMD as their bone outcome variable. However, the use of aBMD in growing children and adolescents has been generally accepted as an inaccurate measure of bone mass accretion because it depends on bone size and body growth factors (Prentice *et al.*, 1994). The increase in aBMD reported in these studies may have been related to differences in bone size between individuals rather than to vitamin D status. Hence, there is still uncertainty about

the importance of vitamin D deficiency during childhood and adolescence on final bone mass at maturity.

There is limited data about the influence of vitamin D status on bone growth in children and adolescents of non-Caucasian origin. In a population-based study of Chinese adolescent girls aged 15 years, adequate vitamin D status of at least 50 nmol/l was found to be associated with a significant increase in size-adjusted BMC of the total body, and distal and proximal forearm compared to those with poor vitamin D status (Foo *et al.*, 2009b). These relationships persisted after adjusting for stage of pubertal development, dietary intake of calcium and vitamin D, and total time spent in physical activity thus demonstrating that these variables did not influence the changes in bone measurements in relation to vitamin D status. In contrast, there were no significant differences in bone area as an indicator of bone size at the skeletal sites measured between the three groups of vitamin D status. These findings were no different from those of Kristinsson *et al.* (1998) where adolescent girls aged 16 to 20 years, showed a significant positive association between 25(OH)D concentration and total forearm BMD and BMC when it was analyzed according to individual age.

Two recent intervention studies of vitamin D supplementation on bone growth in growing adolescents (El-Hajj Fuleihan *et al.*, 2006; Viljakainen *et al.*, 2006) corroborate the prospective cohort studies. In the study of 212 Finnish pre-pubertal adolescent girls aged between 11 and 12 years who were randomly given different doses of vitamin D supplements of either 200 IU or 400 IU daily for a year, it was found that bone mineral augmentation of the proximal femur was 14% and 17% significantly greater in participants who received 200 IU and 400 IU of vitamin D per day, respectively, compared to control girls (Viljakainen *et al.*, 2006). The second investigation was in Lebanese premenarcheal adolescent girls aged 10 to 17 years, supplemented with either 1,400 IU/wk or 14,000 IU/wk vitamin D3 for one-year. They had significantly increased in bone mineral content and bone area of the total hip as compared to the controls (El-Hajj Fuleihan *et al.*, 2006). These findings are further supported by a recent meta-analysis of six randomized-controlled trials of vitamin D supplementation in 541 apparently healthy children and adolescents aged 1 month to <20 years, whose were supplemented for vitamin D for at least three months, showing a significant effects on BMC of the total body and BMD of the lumbar spine, especially among those children and adolescents whose had low vitamin D status of blood (25(OH)D of <35 nmol/l) (Winzenberg *et al.*, 2011).

Most of the published reports investigating the influence of vitamin D status on bone mass in children and adolescents focused on BMC and/or aBMD, assessed by DXA as the endpoint bone variable. This assessment method does not measure bone geometry and structural properties of the skeleton and only provides a two-dimensional rather than a more complex three-dimensional analysis of the bones measured. There are two reports of investigations into the association between vitamin D status and bone geometry. One of these was with Caucasian pre-pubertal girls aged 10 to 12 years (Cheng *et al.*, 2003) and the other was with Hispanic and Caucasian post-pubertal females aged 16 to 22 years (Kremer *et al.*, 2009). The study by Cheng *et al.* (2003) using peripheral quantitative computed tomography, found that girls with vitamin D deficiency, defined

as 25(OH)D levels ≤25 nmol/l, had significantly lower cortical volumetric BMD in the distal radius compared with subjects with higher vitamin D status. By contrast, however, no significant differences were found between vitamin D status and any BMC or BMD measurements for the total body, femur and lumbar spine, measured by DXA device (Cheng *et al.*, 2003). The authors concluded that when only DXA was used to determine the influence of vitamin D status on bone mass, there could be a misinterpretation of the role of vitamin D on bone mass accretion. When peripheral quantitative computed tomography was used to measure volumetric bone density, vitamin D status was found to influence the results (Cheng *et al.*, 2003). On the other hand, no significant association was found between the adequacy of vitamin D status and bone growth as measured by either computed tomography or DXA methods (Kremer *et al.*, 2009).

Although it is well established that low vitamin D status in postmenopausal and elderly women is considered as a major risk factor associated with increased risk of rapid bone loss and osteoporotic fractures (Dawson-Hughes *et al.*, 2005; Lips, 2001), on the contrary, the precise beneficial effects of vitamin D status on bone mass growth and development in growing children and adolescents is still inconclusive. Interpretation of the effects of vitamin D status and bone mass accretion in growing children and adolescents is complicated and confounded by various biological growth factors such as sexual maturation and changes in the rate of linear growth. The interaction between these variables is complex during this rapid growth period (Heaney *et al.*, 2000; Saggese *et al.*, 2002). For instance, there is a strong confounding effect of puberty on skeletal modeling and remodeling, where plasma calcium and phosphate concentrations may be affected by factors other than vitamin D status (Kristinsson *et al.*, 1998; Saggese *et al.*, 2002). This suggests that these biological variations may confound the independent influence of vitamin D on bone mass accretion. In the study of Chinese girls, the significant association between vitamin D status and bone mass remained unchanged, even after adjusting for pubertal stage (Foo *et al.*, 2009b). This is not surprising since most of the subjects were at a post-pubertal stage of development, where changes in sex hormones are less pronounced compared to those in subjects at an earlier stage of puberty (Saggese *et al.*, 2002).

The mechanism by which adequate vitamin D status leads to higher bone mass in healthy growing children and adolescents is not clearly elucidated. However, based on the current published evidence available, it is suggested that the increased bone mass with adequate vitamin D status may be in part a consequence of lower secretion of PTH, followed by a decrease in the rates of bone remodeling, as indicated by the biochemical markers for bone formation and resorption (Foo *et al.*, 2009b; Hill *et al.*, 2010; Outila *et al.*, 2001).

5.3 Effects of adequate vitamin D levels on muscular strength in children and adolescents

It is well documented that long term severe vitamin D deficiency can cause rickets. Rickets is characterized as a failure of mineralization of the organic matrix of bone particularly at the epiphyseal growth plate with deformities of the skeleton, enlargement of the joints of the long

bones and rib cage, curvature of the spine and thighs. Rickets also includes muscle spasms, weakness and atrophy in children, however, there is limited evidence of the effects of vitamin D deficiency and/or insufficiency on muscle functions in growing children and adolescents. A significant positive influence of adequate vitamin D status on muscle strength has been found in a representative population-based study of Chinese post-pubertal adolescent girls aged 15 years, in which adolescent girls with adequate 25(OH)D levels of more than 50 nmol/l showing significantly greater handgrip muscle strength compared with those of lower vitamin D status. This relationship was independent of pubertal growth parameter, body size, level of physical activity and dietary calcium and vitamin D intakes (Foo *et al.*, 2009b). This finding is further supported by another study of 99 post-menarcheal girls aged between 12 and 14 years, also showing a significant positive association between serum 25(OH)D concentration and muscle power and force of the lower limb, assessed by the jumping velocity, height and power, the Esslinger Fitness Index and maximum voluntary force (Ward *et al.*, 2009). Adolescent girls with low 25(OH)D concentrations generated less muscle power, and so jump height and velocity were lower compared to those with higher vitamin D status (Ward *et al.*, 2009), suggesting that muscle contractility is affected by vitamin D status.

The observations from cross-sectional studies are further supported by a recent randomized-controlled trial of vitamin D supplementation on musculoskeletal health assessment in postmenarchal girls aged 12 to 14 years-old with low baseline serum 25(OH)D concentrations of 18 nmol/l, which also found that a significant increase in muscle function, as measured by an efficiency of movement of the lower limb in adolescent girls whose were supplemented with 4 doses of 150,000 IU vitamin D2 in a year compared to the untreated participants (Ward *et al.*, 2010). In addition, there was an significant interaction between baseline serum 25(OH)D concentration and response to vitamin D supplementation for muscle jump velocity. On the contrary, not such significant positive effect of vitamin D supplements on bone mass was found (Ward *et al.*, 2010). These findings in adolescents are comparable to those in postmenopausal women and elderly populations where higher vitamin D status, as assessed by a blood 25(OH)D levels was significantly associated with increased muscle strength and improved lower extremity function (Bischoff-Ferrari, 2012; Bischoff *et al.*, 2003; Dawson-Hughes *et al.*, 1995). In the elderly this improved muscular function and strength may lower the risk of falls and consequent osteoporotic fractures. However, more vitamin D is needed compared to protection against bone loss (Bischoff-Ferrari, 2012).

Until recently, the mechanism of action of higher vitamin D status on greater muscle strength levels and muscle function is not well elucidated in children and adolescents. However, there are several plausible explanations on such positive effect of adequate vitamin D status on muscle. One possible mechanism may involve a direct role of vitamin D (either as $1,25(OH)_2D$ or 25(OH)D) in muscle action. Although such an effect has not been shown, there has been much research on the molecular action of vitamin D in muscle through different pathways involved in genomic and non-genomic actions (Hazell *et al.*, 2012). It has been demonstrated that a direct physiological role of vitamin D receptor in muscle development, in which $1,25(OH)_2D$ stimulates the growth and proliferation of muscle cells and regulates intracellular calcium in muscle cells (Boland, 1986;

Boland *et al.*, 1995) and it is also suggested that the absence of vitamin D receptor or unbound vitamin D receptor in low vitamin D status may produce abnormal muscle growth (Hazell *et al.*, 2012). It may be that vitamin D has a role in modifying the transport of calcium in muscle cells through separate non-genomic mechanisms or it may have a role quite independent of that. For instance, low vitamin D status may result in fatigue and diminished muscle oxygenation, and consequently may affect muscle strength and function, and give rise to muscle weakness (Boland, 1986). On the other hand, a separate observation that 25(OH)D is apparently stored in muscle in newborn rats may indicate a special role of muscle in vitamin D homeostasis, which is quite independent of any role of vitamin D in muscle function (Clements and Fraser, 1988). Therefore, more studies are required to identify the mechanisms for these interactions. Such information is much needed because the development of optimum muscle strength has an important influence on bone mass accrual and also for the maintenance of bone mass integrity during this critical period of growth. For instance, several studies in children and adolescents found that increases in muscular strength were significantly associated with both changes in bone mass (Foo *et al.*, 2007) and also changes in bone geometry, and hence the structural properties of bone, independent of the bone mass properties (Haapasalo *et al.*, 1998). This is compatible with the functional model of bone development, described as the mechanostat hypothesis, in which muscle and bone together form a functional unit, so that greater muscle strength leads to increased bone strength and mass (Frost and Schonau, 2000).

5.4 Recommended daily intake of vitamin D for children and adolescents

The DRI panel for calcium, phosphorus, magnesium, vitamin D and fluoride was first established in 1997 to provide intake recommendations for Americans and Canadians (Institute of Medicine, 1997). At that time, an adequate intake was set for vitamin D as there was not enough data to set an RDA (Weaver and Fleet, 2004). At that time, vitamin D was thought to be mainly acquired by solar irradiation of skin. In 1997, adequate intake for vitamin D was set at 200 IU for infants, children and adolescents, regardless of sunlight exposure, age, degree of skin pigmentation, geographic region and gender. The upper levels were set at 1000 IU for infants and 2,000 IU per day (50 μg/day) for all other age groups. The upper level was set to prevent hypervitaminosis D when serum 25(OH)D concentrations are in excess of 400 nmol/l. Since 1997, there has been debate about whether the adequate intake level for vitamin D is really 'adequate' and ideal for optimal bone health (Dawson Hughes *et al.*, 2005; Vieth, 1999). Since then, progress was made toward establishing the vitamin D intake needed to exert significant effects on bone health. The revised DRI values included an RDA of 600 IU for vitamin D in 2011 (Institute of Medicine, 2011) (Table 5.1). This RDA for vitamin D for growing children and adolescents is three-fold higher than the first recommendation set in 1997. The difference is in the intended level of 25(OH)D thought necessary in order to promote appropriate bone growth; in 1997 the level of 25(OH)D was 25 nmol/l while in 2011 it is 50 nmol/l. DRI values pertain to healthy individuals, while unhealthy children and adolescents may need more vitamin D, for whatever reason may undermine health, from overweight/obesity to malabsorption disorders (Holick *et al.*, 2011). The Endocrine Society has made vitamin D recommendations for 'unhealthy' children and adolescents, as shown in

Table 5.1. In contrast to the DRI values for RDAs, the Endocrine Society has indicated a need to achieve a level of 25(OH)D at or above 75 nmol/l.

5.5 Dietary sources of vitamin D

There are two forms of dietary vitamin D. Vitamin D2 originates from a plant-origin. It is produced by exposure of the ergosterol present in fungi and yeasts to ultraviolet-B radiation. Vitamin D3 is the natural form produced by the cutaneous synthesis by exposure to sunlight in the body in most animals. In general, food sources of vitamin D are extremely limited. The only food sources relatively rich in vitamin D are some oily fish, fish liver oils, and egg yolk. In some developed countries such as Canada and the USA, foods such as breakfast cereals, fruit juices, margarine, and some dairy products are fortified with vitamin D (Calvo *et al.*, 2004). Dietary supplements containing vitamin D in amounts that will increase vitamin D status may also be consumed in these countries (Whiting *et al.*, 2011).

Table 5.1. Dietary reference recommended intake for vitamin D for children and adolescents of healthy populations from the US Institute of Medicine (IOM)[1] and populations at risk of vitamin D deficiency from the endocrine practice guidelines committee.[2]

	IOM (2011) RDA[3]	Endocrine practice guidelines committee (2011) Daily requirement
Children		
1-3 years	600 IU	600-1000 IU
4-8 years	600 IU	600-1000 IU
Males and females		
9-18 years	600 IU	600-1000 IU
19-30 years	600 IU	1,500-2,000 IU

[1] New revised recommended vitamin D intakes from the IOM (2011).

[2] Recommended vitamin D intake for children and adolescents at risk for vitamin D deficiency from the endocrine practice guidelines committee (Holick *et al.*, 2011).

[3] Conversion factor: 1 µg vitamin D3 = 40 IU.

5.6 Conclusions

Low vitamin D status, is not only a biochemical abnormality, but is also associated with physiological, pathological and clinical signs of vitamin D deficiency, along with secondary hyperparathyroidism, and increased bone remodeling, which could have long-term detrimental effects on skeletal development and growth. Moreover, low vitamin D levels also associated with impairment of optimal muscle function in children and adolescents, in which these conditions would therefore compromise bone mass accretion and muscular strength and function during the growing years. It is therefore, an adequate nutritional vitamin D status during growth is important for the maintenance of musculoskeletal health, which significantly could contribute to attaining optimal peak bone mass in growing children and adolescents. It is strongly recommended that children and adolescent should participate in intervention programs to combat vitamin D deficiency. Several preventive strategies to maintain optimum nutritional vitamin D status, such as promoting the production of cutaneous vitamin D through sunlight exposure and intake of vitamin D rich foods such as milk or foods fortified with vitamin D. On the other hand, longitudinal studies with large growing populations warrant further investigation in order to determine what the optimal serum 25(OH)D concentration might be for maximal bone accretion during the time of rapid growth and also to identify the optimal levels of vitamin D intake in relation to greater bone mineralization and physical growth in children and adolescents.

References

Arabi, A., El Rassi, R. and El-Hajj Fuleihan, G., 2010. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nature Reviews Endocrinology 6, 550-561.

Bailey, D.A., McKay, H.A., Mirwald, R.L., Crocker, P.R.E. and Faulkner, R.A., 1999. The University of Saskatchewan Bone Mineral Accrual Study: a six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children. Journal of Bone and Mineral Research 14, 1672-1679.

Bischoff, H.A., Stahelin, H.B., Dick, W., Akos, R., Knecht, M., Salis, C., Nebiker, M., Theiler, R., Pfeifer, M., Begerow, B., Lew, R.A. and Conzelmann, M., 2003. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. Journal of Bone and Mineral Research 18, 343-351.

Bischoff-Ferrari, H.A., 2012. Relevance of vitamin D in muscle health. Reviews in Endocrine and Metabolic Disorders 13, 71-77.

Boland, R., 1986. Role of vitamin D in skeletal muscle function. Endocrine Reviews 7, 434-448.

Boland, R., de Boland, A.R., Marinissen, M.J., Santillan, G., Vazquez, G. and Zanello, S., 1995. Avian muscle cells as targets for the secosteroid hormone 1,25-dihydroxy-vitamin D3. Molecular and Cellular Endocrinology 114, 1-8.

Calvo, M.S., Barton, C.N. and Whiting, S.J., 2004. Vitamin D fortification in the US and Canada: current status and data needs. American Journal of Clinical Nutrition 80, 1710S-1716S.

Cheng, S., Tylavsky, F., Kroger, H., Karkkainen, M., Lyytikainen, A., Koistinen, A., Mahonen, A., Alen, M., Halleen, J., Vaananen, K. and Lamberg-Allardt, C., 2003. Association of low 25-hydroxyvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls. American Journal of Clinical Nutrition 78, 485-492.

Clements, M.R. and Fraser, D.R., 1988. Vitamin D supply to the rat fetus and neonate. Journal of Clinical Investigation 81, 1768-1773.

Cummings, S.R. and Melton, L.J III., 2002. Epidemiology and outcomes of osteoporotic fractures. Lancet 359, 1761-1767.

Dawson-Hughes, B., Harris, S.S., Krall, E.A., Dallal, G.E., Falconer, G. and Green, C.L., 1995. Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. American Journal of Clinical Nutrition 61, 1140-1145.

Dawson-Hughes, B., Heaney, R.P., Holick, M.F., Lips, P., Meunier, P.J. and Vieth, R., 2005. Estimates of optimal vitamin D status. Osteoporosis International 16, 713-716.

Docio, S., Riancho, J.A., Perez, A., Olmos, J.M., Amado, J.A. and Gonzallez-Macias, J., 1998. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis preventing strategies. Journal of Bone and Mineral Research 13, 544-548.

El-Hajj Fuleihan, G., Nabulsi, M., Choucair, M., Salamoun, M., Shahine, CH., Kizirian, A. and Tannous, R., 2001. Hypovitaminosis D in healtly schoolchildren. Pediatrics 107, E53.

El-Hajj Fuleihan, G., Nabulsi, M., Tamim, H., Maalouf, J., Salamoun, M., Khalife, H., Choucair, M., Arabi, A. and Vieth, R., 2006. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. Journal of Clinical Endocrinology and Metabolism 91, 405-412.

Foo, L.H., Zhang, Q., Zhu, K., Ma, G., Greenfield, H. and Fraser, D.R, 2007. Influence of body composition, muscle strength, diet and physical activity on total body and forearm bone mass in Chinese adolescent girls. British Journal of Nutrition 98, 1281-1287.

Foo, L.H., Zhang, Q., Zhu, K., Ma, G., Hu, X., Greenfield, H. and Fraser, D.R., 2009b. Low vitamin D status has an adverse influence on bone mass, bone turnover, and muscle strength in Chinese adolescent girls. The Journal of Nutrition 139, 1002-1007.

Foo, L.H., Zhang, Q., Zhu, K., Ma, G., Trube, A., Greenfield, H. and Fraser, D.R., 2009a. Relationship between vitamin D status, body composition and physical exercise of adolescent girls in Beijing. Osteoporosis International 20, 417-425.

Fraser, D.R., 1995. Vitamin D. Lancet 345, 104-107.

Fraser, D.R., 2004. Vitamin D-deficiency in Asia. Journal of Steroid Biochemistry and Molecular Biology 89-90, 491-495.

Frost, H.M. and Schonau, E., 2000. The 'muscle-bone unit' in children and adolescents: a 2000 overview. Journal of Pediatric Endocrinology and Metabolism 13, 571-590.

Gannage-Yared, M.H., Chemali, R., Yaacoub, N. and Halaby, G., 2000. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. Journal of Bone and Mineral Research 15, 1856-1862.

Gordon, C.M, DePeter, K.C., Feldman, H.A., Grace, E. and Emans, S.J, 2004. Prevalence of vitamin D deficiency among healthy adolescents. Archives of Pediatrics and Adolescent Medicine 158, 531-537.

Guillemant, J., LeTaupin, H.T., Taright, N., Alemandou, A., Peres, G. and Guillemant, S., 1999. Vitamin D status during puberty in French healthy male adolescents. Osteoporosis International 10, 222-225.

Haapasalo, H., Kannus, P., Sievänen, H., Pasanen, M., Uusi-Rasi, K., Heinonen, A., Oja, P. and Vuori, I., 1998. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. Journal of Bone and Mineral Research 13, 310-319.

Hatun, S., Islam, O., Cizmecioglu, F., Kara, B., Babaoglu, K., Berk, F. and Gokalp, A.S., 2005. Subclinical vitamin D deficiency is increased in adolescent girls who wear concealing clothing. The Journal of Nutrition 135, 218-222.

Hazell, T.J., DeGuire, J.R. and Weiler, H.A., 2012. Vitamin D: an overview of its role in skeletal muscle physiology in children and adolescents. Nutrition Reviews 70(9), 520-533.

Heaney, R.P., Abrams, S., Dawson-Hughes, B., Looker, A.C., Marcus, R., Matkovic, V. and Weaver, C,M., 2000. Peak bone mass. Osteoporosis International 11, 985-1009.

Hill, T.R., Cotter, A.A., Mitchell, S., Boreham, C.A., Dubitzky, W., Murray, L., Strain, J.J., Flynn, A., Robson, P.J., Wallace, J.M., Kiely, M. and Cashman, K.D., 2010. Vitamin D status and parathyroid hormone relationship in adolescents and its association with bone health parameters: analysis of the Northern Ireland Young Heart's Project. Osteoporosis International 21, 695-700.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H., Weaver, C.M. and the Endocrine Society, 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism 96, 1911-1930.

Institute of Medicine (IOM)., 1997. Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academies Press, Washington, DC, USA.

Institute of Medicine (IOM)., 2011. Dietary reference intakes for calcium and vitamin D. National Academies Press, Washington, DC, USA, pp. 362.

Johnell, O. and Kanis, J.A., 2004. An estimate of the worldwide prevalence, mortality and disability associated with hip fracture. Osteoporosis International 15, 897-902.

Jones, G. and Dwyer, T., 1998. Bone mass in prepubertal children: gender differences and the role of physical activity and sunlight exposure. Journal of Clinical Endocrinology and Metabolism 83, 4274-4279.

Kremer, R., Campbell, P.P., Reinhardt, T. and Gilsanz, V., 2009. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. Journal of Clinical Endocrinology and Metabolism 94, 67-73.

Kristinsson, J.O., Valdimarsson, O., Sigurdsson, G., Franzson, L., Olafsson, I. and Steingrimsdottir, L., 1998. Serum 25-hydroxyvitamin D levels and bone mineral density in 16-20 years-old girls: lack of association. Journal of Internal Medicine 243, 381-388.

Lehtonen-Veromaa, M., Mottonen, T., Nuotio, I., Irjala, K. and Viikari, J., 2002b. The effect of conventional vitamin D(2) supplementation on serum 25(OH)D concentration is weak among peripubertal Finnish girls: a 3-y prospective study. European Journal of Clinical Nutrition 56, 431-437.

Lehtonen-Veromaa, M.K., Mottonen, T.T., Nuotio, I.O., Irjala, K.M., Leino, A.E. and Viikari, J.S., 2002a. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. American Journal of Clinical Nutrition 76, 1446-1453.

Lips, P., 2001. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine Reviews 22, 477-501.

Looker, A.C., Dawson-Hughes, B., Calvo, M.S., Gunter, E.W. and Sahyoun, N.R., 2002. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone 30, 771-777.

Matkovic, V., Jelic, T., Wardlaw, G.M., Ilich, JZ., Goel, P.K., Wright, JK., Andon, M.B., Smith, K.T., Heaney, R.P., 1994. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Journal of Clinical Investigation 93, 799-808.

Outila, T.A., Karkkainen, M.U. and Lamberg-Allardt, C.J, 2001. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. American Journal of Clinical Nutrition 74, 206-210.

Prentice, A., Parsons, T.J. and Cole, T.J., 1994. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. American Journal of Clinical Nutrition 60, 837-842.

Saggese, G., Baroncelli, G.I. and Bertelloni, S., 2002. Puberty and bone development. Best Practice and Research Clinical Endocrinology and Metabolism 16, 53-64.

Vieth, R., 1999. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. American Journal of Clinical Nutrition 69, 842-856.

Viljakainen, H.T., Natri, A.M., Kärkkäinen, M., Huttunen, M.M., Palssa, A., Jakobsen, J., Cashman, K.D., Mølgaard, C. and Lamberg-Allardt, C., 2006. A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. Journal of Bone and Mineral Research 21, 36-44.

Ward, K.A., Das, G., Berry, J.L., Roberts, S.A., Rawer, R., Adams, J.E. and Mughal, M.Z., 2009. Vitamin D status and muscle function in post-menarchal adolescent girls. Journal of Clinical Endocrinology and Metabolism 94, 559-563.

Ward, K.A., Das, G., Roberts, S.A., Berry, J.L., Adams, J.E., Rawer, R. and Mughal, M.Z., 2010. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. Journal of Clinical Endocrinology and Metabolism 95, 4643-4651.

Weaver, C.M. and Fleet, J.C., 2004. Vitamin D requirements: current and future. American Journal of Clinical Nutrition 80, 1735S-1739S.

Whiting, S.J, Langlois, K., Vatanparast, H., and Greene-Finestone, L., 2011. Vitamin D status of Canadians as measured by plasma 25-hydroxy vitamin D levels in the 2007-2009 Canadian Community Health Survey. American Journal of Clinical Nutrition. 94, 128-135.

Willett, A.M., 2005. Vitamin D status and its relationship with parathyroid hormone and bone mineral status in older adolescents. Proceedings of the Nutrition Society 64, 193-203.

Winzenberg, T., Powell, S., Shaw, K.A. and Jones, G., 2011. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. British Medical Journal 342, c7254.

World Health Organizatio (WHO), 1994. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Technical Report Series. World Health Organization, Geneva.

Key facts

- Our body can synthesize vitamin D from beneath the skin when exposed to ultraviolet light.
- 25-hydroxyvitamin D (25(OH)D) is the main reservoir of vitamin D in our body.
- 1,25-dihydroxyvitamin D is the active form of vitamin D and also a hormone.
- Hypovitaminosis D is defined as the level of 25(OH)D less than 30 ng/ml.
- Prevalence of hypovitaminosis D range from 40 to 70% in postmenopausal women and increases with age.
- The elderly above 70 years old are capable of synthesizing less than 30% vitamin D compared to the young when equally exposed to the sun.

Summary points

- Vitamin D plays a major role in calcium and phosphorus homeostasis.
- Vitamin D also plays a major role in muscle functions.
- Hypovitaminosis D is more prevalent in postmenopausal women and increases with age.
- Usually, hypovitaminosis D presents with no symptoms accept for some muscle fatigue and low bone mineral density.
- Replacement of vitamin D can improve muscle function and reduce the risk of falls in subjects that have hypovitaminosis D.

6. Hypovitaminosis D and muscle strength

T. Songpatanasilp
Division of Orthopaedic Oncology and Bone Metabolism, Department of Orthopaedics, Phramongkutklao College of Medicine, 315 Rajavithi Rd., Rajathavee, Bangkok 10400, Thailand;
thaweesps@yahoo.com

Abstract

Vitamin D plays a major role in calcium and phosphorus homeostasis. The natural forms of vitamin D are 'ergocalciferol' from vegetable food, and 'cholecalciferol' from the synthesis beneath our skin when exposed to ultraviolet light. These two forms of vitamin D have to change into 25-hydroxyvitamin D (25(OH)D), the main reservoir of vitamin D in our body, by hydroxylation in the liver. Then 25(OH)D undergoes further hydroxylation in the kidney to $1\alpha,25(OH)_2D$, the active functioning hormone in our body. $1\alpha,25(OH)_2D$ will interact with vitamin D receptor (VDR) at the cellular level and result in the transcription and translation of many vitamin D dependent proteins. Nowadays we can detect VDR in various tissues which confirms the many biological effects that $1\alpha,25(OH)_2D$ might have, apart from calcium and phosphorus homeostasis. Those unexpected tissues include pancreas, placenta, pituitary, ovary, testis, mammary gland, muscle and heart. Our understanding of vitamin D deficiency syndrome has changed over the past 10 years. Hypovitaminosis D or vitamin D insufficiency refers to the low level of serum 25(OH)D which induces the depletion of tissue levels and the elevation of parathyroid hormone levels. Hypovitaminosis D can be defined as the serum level of 25(OH)D below 30 ng/ml (75 nmol/l). Hypovitaminosis D is more prevalent among postmenopausal women, ranging from 40 to 70% of this population and increasing with age. This high prevalence of hypovitaminosis D causes depletion of muscle function as well as an increased risk of falls in this elderly group. Many studies show that replacement of vitamin D can improve muscle function and reduce the risk of falls in subjects that have hypovitaminosis D.

Keywords: vitamin D insufficiency, vitamin D inadequacy, osteoporotic fractures, myopathy, falls

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_6, © Wageningen Academic Publishers 2013

Abbreviations

1α,25(OH)$_2$D	1α,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
25(OH)D3	25-hydroxycholecalciferol
BMD	Bone mineral density
DBP	Vitamin D binding protein
HDM	Hypovitaminosis D myopathy
PTH	Parathyroid hormone
RXR	Retinoid-X receptor
VDR$^{-/-}$ mice	VDR knockout mice
VDR	Vitamin D receptor
Vitamin D2	Ergocalciferol
Vitamin D3	Cholecalciferol

6.1 Introduction

Vitamin D is very well known for its vital role in controlling the metabolism of calcium and phosphorus in all kinds of vertebrates by working through 3 major organ systems, namely the intestinal, renal and skeletal system. 1α,25(OH)$_2$D, or the active form of vitamin D, functions as a major gear wheel within these systems which might be regarded as one of our body's hormones and is sometimes called the 'D-hormone'. Currently, it is known that 1α,25(OH)$_2$D has various target tissues and organs. We can classify these target tissues into 2 main categories (Demay, 2003): (1) target tissues related to mineral homeostasis including intestine, renal, bone and parathyroid gland are called the 'traditional target tissues'; (2) target tissues unrelated to mineral homeostasis are called the 'non-traditional target tissues'. Advancements in research involving VDR and VDR knockout mouse have vastly broadened our knowledge and understanding of the non-traditional target tissues (Li *et al.*, 1998). For the past 10 years, VDR has been detected within various cells and tissues beyond the bone as well as the tissues related to the mineral metabolism. This suggests 1α,25(OH)$_2$D has a large number of non-traditional functions which have not been clearly investigated, involved with the function of tissues within various systems beyond the skeletal system. There are several lines of evidence supporting the theory that 1α,25(OH)$_2$D might play a role in controlling the proliferation and differentiation of cells and other tissues besides an osteoblast. Moreover, there is activity in immunoregulation, hair follicle cycling, blood pressure regulation, mammary gland development and also the ability to modulate the secretion of insulin and prolactin (Walters, 1992). Several lines of evidence report that muscle cells are one of the important target tissues of vitamin D. It has also been found that muscle cells can express classical VDR (Boland, 2005; Boland *et al.*, 1985; Demay, 2003) as well as demonstrate the non-genomic actions (De Boland *et al.*, 1994; Morelli *et al.*, 1993) of vitamin D within muscle cells which will be described in detail later.

6.2 The vitamin D endocrine system

The vitamin D endocrine system is very important to mineral homeostasis and skeletal integrity as well as to the development and physiology of other tissue systems. The question, therefore, is this: how is 1α,25$(OH)_2$D which is a key functional molecule of this system, created? The source of 1α,25$(OH)_2$D comes from a substance within a group of secosteroids called vitamin D2 which occurs naturally, and also comes from self-synthesis in the skin called vitamin D3. Vitamin D2 is a natural vitamin found in various types of plants, while vitamin D3, which is a precursor of 1α,25$(OH)_2$D3, can be obtained from 2 sources, namely food and most importantly from the synthesis in the skin when exposed to ultraviolet light. Since our body can synthesize vitamin D3 by itself, cholecalciferol does not seem to fit well with the definition of a vitamin (theoretically, a vitamin should be categorized as a vital substance that our body needs but is unable to produce itself). The precursor of vitamin D3 is '7-dehydrocholesterol' in skin, which requires ultraviolet light with frequency of 290-315 nm in sunlight (Holick, 2008) to turn it into vitamin D3. Then vitamin D3 will be bound by DBP and carried to the liver. Vitamin D3 will then be turned into 25(OH)D3 by the enzyme vitamin D-25-hydroxylase. 25(OH)D3, which is a major circulating form of vitamin D within mammals, is transported to the kidney by DBP. 25(OH)D-DBP will be filtered by the glomerulus and reabsorbed via the active endocytotic process by the receptor called 'Megalin' (Holick, 2008) in order to preserve 25(OH)D3 and be conveyed to the renal tubular cells. 25(OH)D3 is turned into 1α,25$(OH)_2$D3 by cytochrome P450 enzymes called 'renal-25(OH)D3-1α-hydroxylase'. 1α,25$(OH)_2$D3, or the active form of vitamin D, is held to be one of our body's hormones for which the kidney is a key endocrine gland. The renal proximal tubular cells will create and adjust this hormone according to the physiological stimulation of the body, thus this whole system is known as the 'vitamin D endocrine system' (Figure 6.1). 1α-hydroxylase will be stimulated from the conditions of low serum calcium, low serum phosphate and high serum PTH. In contrast, 1α-hydroxylase is negatively regulated by the increased level of 1α,25$(OH)_2$D3 itself (Holick, 2008). The inactivation and catabolism of 1α,25$(OH)_2$D3 derives from the enzyme 25-hydroxyvitamin D3-24-hydroxylase which will hydroxylase 25(OH)D3 and 1α,25$(OH)_2$D3 and turn it into 24,25$(OH)_2$D3 and 1,24,25$(OH)_3$D3 respectively (Holick, 2008), before being degraded and excreted from the body. This 24OHase system is regarded as the system which helps to keep the levels of 1α,25$(OH)_2$D3 stable.

1α,25$(OH)_2$D3 plays an important role in controlling various bodily systems by having the genomic transcription mechanism bound mainly to one of the nuclear receptors called VDR. VDR is one of the members of the classical endocrine nuclear receptor super-family as well as thyroid hormone receptors, receptors of sex hormone (estrogen receptor, progesterone receptor, etc.) and receptors of corticosteroid hormone. Upon binding to 1α,25$(OH)_2$D3 the VDR will combine with RXR. This complex acts as a transcription factor regulating the transcription of various important genes within our body. Especially in the skeleton system, there are calcium binding proteins that regulate genes encoding proteins associated with calcium metabolism, genes encoding osteocalcin and osteopontin, as well as the genes that encode various proteins related with osteoblast proliferation and differentiation such as Runx2, COL1A1, RANKL, etc. (Atkins *et al.*, 2005). This mechanism is commonly known as the 'genomic pathway'. Moreover,

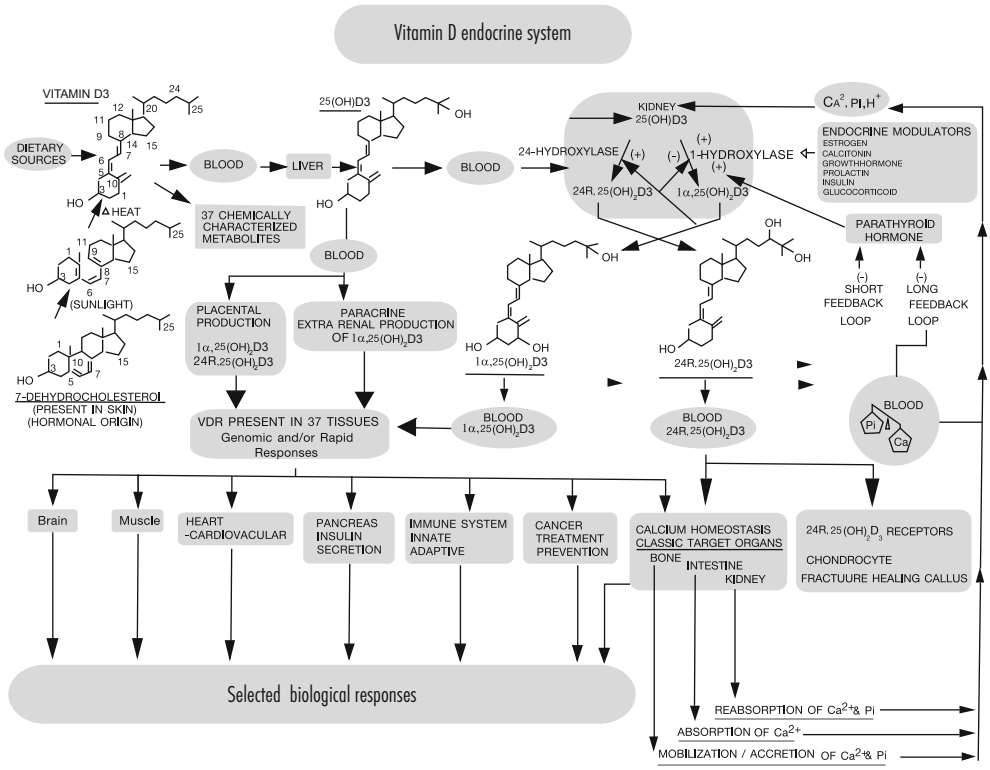

Figure 6.1. The vitamin D endocrine system: from photosynthesis and dietary sources to the active form of vitamin D, 1,25(OH)$_2$vitamin D3, which controls a wide range of tissue homeostasis.

not only can 1α,25(OH)$_2$D3 function without the binding process to the nuclear receptor; it is also capable of stimulation at the plasma membrane through the transmembrane receptor and forwarding signals internally via the signal transduction pathway. This process is similar to growth factors and other peptide hormones, and is called the 'non-genomic pathway' (De Boland and Nemere, 1992; Nemere and Farach-Carson, 1998).

6.3 The vitamin D receptor

The idea that vitamin D may act as a type of steroid hormone appeared in 1968 (Norman, 1968). Later 1α,25(OH)$_2$D was discovered to be an active hormonal form of vitamin D (Holick *et al.*, 1971; Lawson *et al.*, 1971; Norman *et al.*, 1971). The fact that 1α,25(OH)$_2$D is a lipophilic molecule and localized in the nucleus of target tissue, as well as the fact that vitamin D response is often inhibited by transcriptional inhibitors, have resulted in theories that there might be a protein which acts as a vitamin D nuclear receptor similar to other steroid hormones. The VDR itself was successfully cloned for the first time in poultry intestinal tissues (McDonnell *et al.*, 1987). Current-day technologies allow us to detect VDR in various tissues which confirms

the many biological effects that 1α,25$(OH)_2$D might have, apart from calcium and phosphorus homeostasis. These tissues include pancreas, placenta, pituitary, ovary, testis, mammary gland, muscle and heart. Furthermore, other cultured cell lines, including intestinal cells, kidney cells, skin cells, fibroblastic, chondrocytic, osteoblastic, myoblastic, hematopoietic and lymphopoitic cells, all express VDR entirely (Pike and Shevde, 2005).

VDR belongs to a member of ligand-activated transcription factors in the nuclear receptor superfamily. 1α,25$(OH)_2$D upon binding to VDR will initiate several continuous reactions, beginning by forming itself into a heterodimer with RXR. Ligand VDR-RXR heterodimer then functions as an active transcription factor binding with high affinity to vitamin D response elements of the target genes which then induces the transcription (Sutton and McDonald, 2003). The process of ligand activated transcription is actually far more complicated and will be discussed in detail elsewhere in this book. The facts show that all the responses of each cell type to 1α,25$(OH)_2$D are different. In addition to the previously mentioned level of VDR as a determinant, more important factors are the quantities and types of co-modulators which vary within each cell type. There are 2 major co-modulators comprising the co-activators which promote the transcription upon binding to ligand VDR-RXR heterodimer complex, as well as the co-repressors which conversely inhibit the transcription when binding to the ligand VDR-RXR heterodimer complex (Sutton and McDonald, 2003). These co-modulator molecules are the main factors for determining the specific cellular response of each individual's tissue type to the steroid hormone or what we call the 'selective receptor modulator concept'.

6.4 Vitamin D deficiency and hypovitaminosis D

Age, location, time of day, season and skin color are all factors which affect the synthesis of vitamin D via skin. It has been shown that the elderly are more at risk of vitamin D deficiency syndrome for many reasons. When compared to the general youth, the over-70s are capable of synthesizing less than 30% vitamin D when equally exposed to the sun (Holick *et al.*, 1989). Furthermore, the elderly might consume food with low levels of vitamin D or they might have worse intestinal absorption when compared to younger people. The other important reason could be impaired hydroxylation in the liver and the kidney; functional problems of these 2 organs are often found among the elderly. The level of 25(OH)D in serum, which is the precursor of 1α,25$(OH)_2$D, will be 1000 times higher than 1α,25$(OH)_2$D level and 25(OH)D acts as the storage source of 1α,25$(OH)_2$D. Therefore, it is preferable to measure the serum 25(OH)D level rather than the vitamin D level in our body.

Our understanding of vitamin D deficiency syndrome has changed over the past 10 years. Vitamin D deficiency syndrome is determined by low levels of serum 25(OH)D, until the patient shows signs of a lack of vitamin D such as hypocalcemia, secondary hyperparathyroidism, muscle pain and weakness, sarcopenia, bone pain, insufficiency fractures, osteomalacia, etc. At this stage the serum 25(OH)D level often remains very low. Most laboratories use the minimum value of the normal serum 25(OH)D level of 6-20 ng/ml (15-50 nmol/l) as a criterion (Binkley *et al.*,

2004). Nonetheless, there are some discrepancies in differentiating 'vitamin D insufficiency' from 'vitamin D deficiency' as some researchers believe both syndromes have different effects as regards calcium homeostasis and bone metabolism. The term 'vitamin D sufficiency' is defined as the serum 25(OH)D3 level which has absolutely no effect on calcium homeostasis and signifies the normal condition. Whereas 'vitamin D insufficiency', which is sometimes called 'hypovitaminosis D' (Zittermann, 2003) or 'vitamin D inadequacy' or 'mild vitamin D deficiency', refers to the low level of serum 25(OH)D which induces the depletion of tissue levels and the elevation of PTH level. Hypovitaminosis D usually has no distinct clinical signs other than possibly some fatigue or aches and pains, and the serum calcium level still remains within the normal range while the serum 25(OH)D level which enhances the serum PTH level is different among each population group. The study by Malabanan (1998) designated the serum 25(OH)D level at 20 ng/ml (50 nmol/l) as a threshold. Also, Chapuy *et al.* (1997) found that the serum PTH level started to increase when the serum 25(OH)D3 level was lower than 31 ng/ml (78 nmol/l). Furthermore, Heaney (2003a,b) found that a serum 25(OH)D3 level of 32 ng/ml (80 nmol/l) did not increase of serum PTH level. Contrary, in a study in North Eastern Thailand (Soontrapa *et al.*, 2001) conducted in elderly women (average age 69 years old) found that the serum PTH level started to increase when the serum 25(OH)D level was lower than 35 ng/ml (87.5 nmol/l), and significantly increased when the serum 25(OH)D level was lower than 30 ng/ml (75 nmol/l). It is still somewhat controversial to use the serum 25(OH)D value as an indicator of a patient's conditions, especially when the clinical criteria are used to judge whether the symptoms will occur or not, which are often variable in each person. The point at which the serum PTH level starts to increase is still unclear due to the difference in threshold within each population. Moreover, the value of serum 25(OH)D which is regarded as a normal value for each laboratory is still controversial. On average, the prevalent use of normal serum 25(OH)D values is much lower than it should be due to the overestimation of vitamin D toxicity state (Vieth, 1999, Holick, 2003).

Figure 6.2. shows the ranges of the serum 25(OH)D values for the classification of the vitamin D condition. The criteria for assigning the serum 25(OH)D values to different classes are as follows:

- *Vitamin D deficiency: less than 20 ng/ml (50 nmol/l).* The serum 25(OH)D levels below 20 ng/ml (50 nmol/l) significantly determine the condition of vitamin D deficiency. However, some authorities believe that this value is too low. Heaney (2003c) and Holick (2003) still affirm that the serum 25(OH)D level should be higher than 30 ng/ml (75 nmol/l) in order to have no symptoms.
- *Vitamin D insufficiency: less than 30 ng/ml (75 nmol/l).* According to Zittermann (2003), the condition of hypovitaminosis D which started to cause a decrease in vitamin D tissue levels and an increase in serum PTH levels would correlate with the serum 25(OH)D at approximately 30-40 ng/ml (75-100 nmol/l). Dawson-Hughes *et al.* (1997a) demonstrated that the PTH level increased when the serum 25(OH)D level was lower than 45 ng/ml (112 nmol/l) among elderly men and women. Kinyamu *et al.* (1998) also conducted the study specifically in elderly women and found that serum PTH level started to increase when the serum 25(OH)D level was lower than 49 ng/ml (122 nmol/l). However, as mentioned before, the studies by Chapuy *et al.* (1997), and Heaney *et al.* (2003a,b), have concluded that the optimal cutoff level of 25(OH)D from which the serum PTH level started to increase should

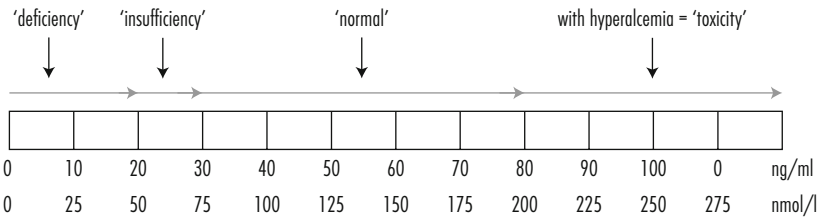

Figure 6.2 The classification of deficient-excessive conditions of vitamin D by the serum 25(OH)D3 levels, based on the current research. (Vasquez, 2004)

be 31-32 ng/ml (77.5-80 nmol/l). We conclude from the above-mentioned study that the serum 25(OH)D level which is classified as vitamin D insufficiency should be around 20-40 ng/ml (50-100 nmol/l). Recent recommendations by several organizations suggest that the normal 25(OH)D level should begin at 30 ng/ml (75 nmol/l) and above (Dawson-Hughes *et al.*, 2005; Hanley *et al.*, 2010; Mithal *et al.*, 2009; National Osteoporosis Foundation, 2008). There are two reasons for setting the low end of the normal range for 25(OH)D at 30 ng/ml (75 nmol/l): (1) studies published in the last years, mostly suggests that levels of PTH rise when levels of 25-hydroxyvitamin D fall below 30 ng/ml (75 nmol/l) (Heaney, 2005; Holick, 2007; Steingrimsdottir *et al.*, 2005); (2) earlier studies suggests that active calcium absorption is optimal when the level of 25(OH)D is 30 ng/ml (75 nmol/l) (Chapuy *et al.*, 1997).

- *Optimal vitamin D status: 30-65 ng/ml (75-160 nmol/l).* Zittermann (2003) stated that the sufficient and normal value of serum 25(OH)D should fall within the range of 40-80 ng/ml (100-200 nmol/l). After having reviewed many of the above-mentioned studies, the lower limit of normal range has been accepted as 30 ng/ml (75 nmol/l) (the normal vitamin D level should not be less than this) whereas the upper limit value is still unclear. But it is clear that the value must be safe from vitamin D excess and vitamin D toxicity. Zittermann (2003) concluded that the serum 25(OH)D level which was safe from any toxicity could rise up to 100 ng/ml. However, according to the study of the safe upper limit, it was found that respondents who only exposed themselves to sunlight could raise the serum 25(OH)D level higher than 80 ng/ml (200 nmol/l). And those who consumed vitamin D via oral supplements up to 10,000 IU daily would raise the serum 25(OH)D level higher than 80 ng/ml (200 nmol/l) with no toxicity found for both cases (Heaney, 2003a). Thus, the use of 65 ng/ml (160 nmol/l) as a criterion would be the most appropriate value to ensure higher safety levels.
- *Vitamin D excess: greater than 80 ng/ml (200 nmol/l) with accompanying hypercalcemia.* As mentioned above, a serum 25(OH)D level higher than 80 ng/ml (200 nmol/l) does not specify an occurrence of toxicity except if the clinical signs of accompanying hypercalcemia appear. Vieth (1999) stated that for hypercalcemia from hypervitaminosis D the serum 25(OH)D level must always be higher than 88 ng/ml (220 nmol/l). Holick (2000) also reported that vitamin D intoxication would not occur until the level was higher than 125 ng/ml (312 nmol/l). Therefore, the criterion expressing the vitamin D excess level should be higher than 80 ng/ml (200 nmol/l), and the vitamin D toxicity level is indicated when are also clinical signs of hypercalcemia.

There are not many studies regarding vitamin D deficiency and hypovitaminosis D in Thailand as it is often believed that a tropical country should have no problem of vitamin D deficiency owing to the abundance of sunlight. The study by Chailurkit *et al.* (1996) in 158 men and women aged between 20-80 years found that the average value was 42 ng/ml (106 nmol/l) and concluded that there was no vitamin D deficiency in Thailand. However, there were only 39 postmenopausal women among volunteers within this study. The study by Soontrapa *et al.* (2001) among 106 Thai elderly women (average age at 69 years) in Khon Kaen province found that the serum PTH level would increase when the serum 25(OH)D level started to decrease by 35 ng/ml (87.5 nmol/l), and it would increase significantly when the serum 25(OH)D level was lower than 30 ng/ml (75 nmol/l). It was shown from this study that at a threshold level of 30 ng/ml (75 nmol/l), 35% would have hypovitaminosis D, but when a threshold level of 35 ng/ml (87.5 nmol/l) was used, 65% would have hypovitaminosis D.

A study by Songpatanasilp *et al.* (2009) measuring the serum 25(OH)D level among 72 elderly women aged above 65 years old (average age 71 years) who all attended the Orthopaedics clinic at Phramongkutklao hospital with various diseases, found that hypovitaminosis D [defined as the level below 30 ng/ml (75 nmol/l)] was detected up to 64%, the same very high level as found by Soontrapa *et al.* (2001). It might be possible that even though vitamin D deficiency is rarely found among the general population, it can be commonly found in some subpopulations, such as very old postmenopausal women (>65 years) who attended the hospital with various kinds of musculoskeletal pain. All the above-mentioned studies in Thailand correspond to other studies carried out in neighboring tropical countries (Gannage-Yared *et al.*, 2000; Ghannam *et al.*, 1999; Goswami *et al.*, 2000). Thus it is possible to conclude that a problem of hypovitaminosis D and vitamin D deficiency exists, and that it is more prevalent than expected in tropical countries.

6.5 Osteomalacic myopathy and hypovitaminosis D myopathy

The symptom of 'osteomalacic myopathy' can often be found in patients with osteomalacic bone disease, and both conditions can be considered major symptoms of vitamin D deficiency muscle weakness. Hypotonia is also found a major symptom of these conditions, sometimes referred to as 'vitamin D deficiency-related myopathy'. The classical condition will be the proximal muscle weakness especially in the areas of extensor, flexor and abductor muscles of hip, and also the flexor, extensor muscles of knee, as well as the accompanying diffuse skeletal pain. Skaria *et al.* (1975) reported the 25 osteomalacia patients who expressed abnormality in a muscle electromyogram which showed the characteristics of both the myopathy and also the decrease in nerve conduction velocity. However, whether the peripheral nerve is involved or not remains controversial. Yoshikawa *et al.* (1979) conducted the muscle biopsy in osteomalacia patients and found atrophy of type-II muscle fibers as well as dilation of interfibrillar spaces which were infiltrated with fat, fibrosis and glycogen granules. These characteristics were different from the neuropathic muscle in which the atrophy was found in both type-I and type-II muscles, and they were also different from immobilization muscle atrophy in which the atrophy was found only in type-I muscle. In circumstances where a sudden movement is required, the type-II muscle

fibers which can contract stronger and faster will function first to prevent falling (McComas, 1996). This helps to explain why patients with vitamin D deficiency, mainly affecting type-II fibers, will have a higher tendency to fall. We have discovered that the myopathy state can be detected before the biochemical signs of osteomalacia are present (Glerup *et al.*, 2000) or even found in falling patients who have a hip fracture even before secondary hyperparathyroidism has occured (Bischoff-Ferrari *et al.*, 2003). We refer to the myopathy state which occurs without other symptoms of vitamin D deficiency as HDM (Glerup and Eriksen, 2005). Such a myopathy state has long been overlooked and misdiagnosed for 2 reasons: (1) the decrease in muscle strength is a phenomenon which gradually and continually occurs whereas the loss of functional ability is not a continual phenomenon; in addition the muscle strength of patients might slowly be wearing down anyway, and patients will only complain if they really feel tired or unable to do what they could do before. (2) the symptoms of HDM are quite unspecific and there are many symptoms of other diseases which are similar to the group of non-specific rheumatic diseases such as polymyalgia, fibromyalgia and psychoneurotic disorders, etc. Recent studies have found that the most distinct symptom of HDM (Glerup and Eriksen, 2005) is a diffuse muscle pain especially in the proximal muscles of lower limbs, arms and muscle around the shoulder which could later develop into deep bone pain, fatigue, inability to walk upstairs, inability to stand up from a chair or lift things (even light objects), and probably peripheral paresthesia, muscle cramps as well as joint pain. All the above-mentioned symptoms will correlate significantly with the serum 25(OH)D but not quite correlate with the serum 1α,25$(OH)_2$D (Glerup, 1999).

A study conducted in elderly patients (65-95 years old) whose 25(OH)D serum level was <12 ng/ml (30 nmol/l) (Bischoff-Ferrari *et al.*, 1999) found a significant correlation between the serum 25(OH)D and the leg extension power. This was in line with a study by Mowe *et al.* (1999) in 349 elderly people (>70 years old) whose serum 25(OH)D level was significantly lower in the group that had less handgrip strength, that were unable to walk upstairs, that undertook no outdoor activities and that had fallen at least once within the past month. Moreover, Geusens *et al.* (1997) conducted an interesting study to explore the relationship between the VDR polymorphism and the muscle function in nonobese women over 70 years old and found the difference between quadriceps muscle strength statistically significant ($P<0.01$) between the group that has bb genotype and BB genotype. A study by Songpatanasilp *et al.* (2009) in Thai postmenopausal women [average age of 71 years old] also demonstrated that the serum 25(OH)D level of respondents was below 30 ng/ml (75 nmol/l). The respondents were randomized and allocated to two groups and the focus group received alfacalcidol (1α-hydroxyvitamin D) combined with calcium. It was shown that the quadriceps muscle strength measured using an isokinetic dynamometer could be improved significantly compared to the other group which received calcium only.

Until now, only two studies have examined whether vitamin D supplementation may have an impact on muscle fiber composition. In a small uncontrolled study, Sorensen *et al.* (1979) obtained muscle biopsies from elderly women after treatment with 1α-hydroxyvitamin D and calcium for 3-6 months. Results showed an increase in relative fiber composition and in fiber area of type II muscle fibers. More recently, a randomized controlled study found that treatment

of elderly stroke survivors with 1000 IU of vitamin D2 daily significantly increased mean type II muscle fiber diameter and percentage of type II fibers over a 2 year period (Sato *et al.*, 2005). There was also a correlation between serum 25(OH)D level and type II muscle fiber diameter both at baseline and after two years of follow-up (Sato *et al.*, 2005). It remains unclear, however, if the increase in type II muscle fiber number is caused by new formation of type II fibers or a transition of already existing fibers from type I to type II. Nevertheless, the effect on muscle caused by vitamin D appeared to be the direct result of vitamin D itself rather than the indirect result of the increase in calcium.

6.6 Genomic and non-genomic effects of vitamin D on muscle

We now recognize that striated muscle cells can express VDR and also find that vitamin D can stimulate the synthesis of various important muscle proteins such as troponin-C and actin in the sarcoplasmic reticulum and the inner membrane of mitochondria by genomic transcription process (Glerup and Eriksen, 2005). Endo *et al.* (2003) conducted study in $VDR^{-/-}$ mice which had distinct clinical signs of vitamin D resistant rickets (i.e. hypocalcemia, hypophosphatemia, secondary hyperparathyroidism and osteopathy). When $VDR^{-/-}$ mice received a high calcium diet [rescue diet] from birth, those mice remained normal until this diet was no longer provided. However, the muscle abnormality was detected in the diseased $VDR^{-/-}$ mice and the $VDR^{-/-}$ mice that were on the rescue diet, showing that such muscle abnormality was independent of the secondary effect from metabolism change. The study in cultured myoblast found that the VDR was necessary in the normal development and gene expression of muscle cells. Endo *et al.* (2003) also found that the abnormality of muscle which occurred as a result of the ligand-dependent action of VDR in the protein transcription was related to muscle cell proliferation and differentiation. In addition, the genomic effects of vitamin D in muscle cells are also related to the synthesis of proteins used in the regulation of muscle cell proliferation and differentiation (Drittanti *et al.*, 1989b), calcium transport (Drittanti *et al.*, 1989a) and phospholipid metabolism (Drittanti *et al.*, 1988). Calcium binding proteins, such as calbindin-D9K, are examples of newly synthesized myoblast proteins expressed by activation of the nuclear VDR (Zanello *et al.*, 1995). Prolonged treatment with $1\alpha,25(OH)_2D$ has been shown to affect the synthesis of certain muscle cytoskeletal proteins which are important in controlling muscle cell functions (Boland *et al.*, 1995). Other $1\alpha,25(OH)_2D$-dependent proteins have been described as calmodulin-binding components of the myoblast cytoskeleton (Brunner and de Boland, 1990). Calmodulin is a calcium-binding protein that regulates several cellular processes including muscle contraction. Experiments in mitotic myoblasts treated with $1\alpha,25(OH)_2D$ revealed increased synthesis of calmodulin (Drittanti *et al.*, 1990). *In vitro* and *in vivo* experiments in chick skeletal muscle have shown that $1\alpha,25(OH)_2D$ regulates muscle calcium uptake by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma. It also regulates the calcium influx via voltage-sensitive calcium channels thereby altering intracellular calcium (Boland, 1986). Modifications in intracellular calcium levels control muscle contraction and relaxation, thus impacting muscle function (Boland *et al.*, 1995, Ebashi and Endo, 1968). $1\alpha,25(OH)_2D$ also appears to play a significant role in the regulation of phosphate metabolism of myoblastic cells (Boland *et al.*, 1995). In skeletal

muscle cells phosphate in the form of ATP or inorganic phosphate is necessary for the structural and metabolic needs of the cell. Exposure to $1\alpha,25(OH)_2D$ stimulates accelerated phosphate uptake and accumulation in these cells.

Apart from the classical genomic effects of vitamin D, also the various non-genomic effects of vitamin D were discovered (Figure 6.3). The definition of non-genomic pathway means that $1\alpha,25(OH)_2D$ could act by binding to the membrane receptor, causing the stimulation of 'second messenger systems' which will transmit the signal to the cytoplasm; this effect is sometimes called the 'fast effect' (Boland, 2005). Such stimulation results in: (1) the activation of Ca^{2+} flows into cells via G-protein-mediated voltage-dependent Ca^{2+} channels by means of the adenylyl cyclase/cAMP/protein kinase A pathway (De Boland *et al.*, 1995) and the PLC/DAG+IP_3 pathway (Morelli *et al.*, 1996). Moreover, the Ca^{2+} release can also be stimulated from the intracellular stores – the store-operated calcium channels; (2) the proliferation and growth of muscle cells by the stimulation of tyrosine phosphorylation of the MAP kinase pathway (Boland, 2005); and (3) induction of rapid translocation of VDR towards the cell membrane via the tyrosine kinase pathway (Boland, 2005).

It has been shown that $1\alpha,25(OH)_2D$ has a significant and direct effect on muscle calcium action as well as the growth and differentiation of muscle cells, whether it is a genomic or non-genomic effect. $1\alpha,25(OH)_2D$ has a similar effect on the muscular system to one of the hormones in this system which performs the control work extremely well. Previously there has not been much interest in this subject, however, recently, we have begun to understand it better.

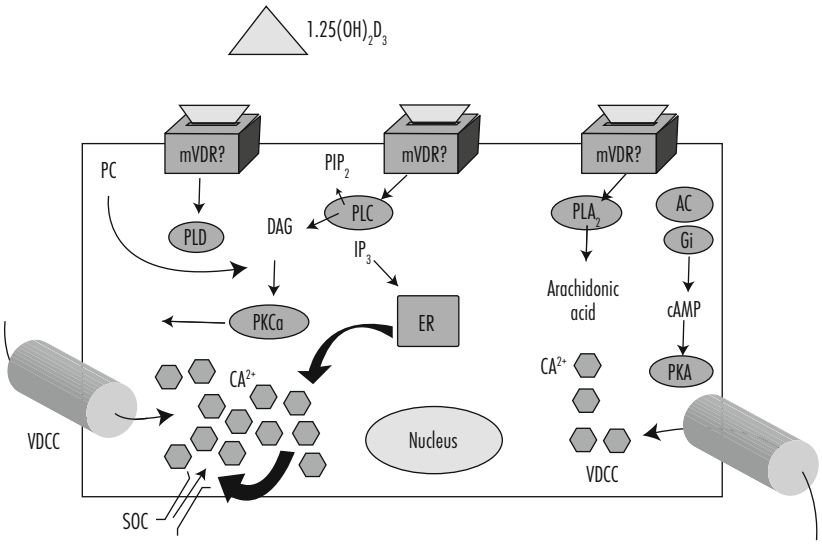

Figure 6.3. Non-genomic action of 1,25(OH)2D3 in the stimulation of membrane vitamin D receptor (mVDR) towards second messenger of protein kinase A (PKA) and C (PKC) pathways (Feldman *et al.*, 2005).

6.7 Vitamin D and falls

Chapuy *et al.* (1992) inspired many researchers on the relationship between muscular effects and vitamin D. The authors conducted the study on 3,270 elderly people who lived in a nursing home showing that the hip fracture incidence could be lowered by 43% in the group that received the 800 IU of vitamin D3 and 1,200 mg of calcium per day compared to the placebo group over a period of 18 months. The study caused great astonishment since there was almost no change in BMD (~2% increase from baseline), and the hip fracture reduction still remained after 3 years. Moreover, the study of Dawson-Hughes *et al.* (1997b) found 58% reduction in non-vertebral fracture in 399 elderly people (>65 years) who received 700 IU of vitamin D supplement and 500 mg of calcium. The idea that vitamin D + calcium could lower the incidence of fractures without increasing BMD might derive from the ability to improve the muscle strength and reduce the risk of falls by vitamin D.

On average, 33% of elderly people fall at least once a year and approximately 6-7% of them experience a hip fracture as a result (Graafmans *et al.*, 1996; Tinetti *et al.*, 1988). As mentioned, vitamin D deficiency and hypovitaminosis D often cause muscle weakness especially in the weight-bearing muscles of lower limbs which are very important to postural balance and walking. More muscle weakness will affect the postural balance in standing, walking and the changing of posture. Body sway and poor functioning of weight-bearing muscles also contribute to falling more easily. Moreover, a reflex to prevent falling as well as a change of posture lower than expected will normally cause elderly people to fall on their back, having the hip, the back and the head hit by the floor, rather than falling forward using their hands to stop the fall.

The study by Mowe *et al.* (1999), as well as the study by Stein *et al.* (1999) on elderly people found a significant correlation between serum 25(OH)D3 and occurrence of falls. A study in a 'falls clinic' in London (Dhesi *et al.*, 2002), with patients that have fallen at least once within 8 weeks attending the clinic, found that up to 32% of patients had vitamin D deficiency (25(OH)D <12 ng/ml), while up to 73% patients had mild vitamin D deficiency (25(OH)D <20 ng/ml). The patient group with serum 25(OH)D3 level less than 12 ng/ml (30 nmol/l) was found to have significantly more accompanying impaired psychomotor function, increased body sway, impaired reaction time and decreased isometric quadriceps muscle strength.

6.8 Therapeutic implications

Chapuy *et al.* (1992) and Dawson-Hughes *et al.* (1997b) demonstrates the significant antifracture efficacy of vitamin D in combination with calcium while being independent of BMD. The fracture reduction is very distinct, especially for hip and non-vertebral fractures. It seems that the anti-fracture efficacy of vitamin D + calcium is not the result of increasing BMD, but possibly the fact that vitamin D + calcium can lower secondary hyperparathyroidism. Alternatively, it can result from the preservation of bone strength, and the decrease in bone turnover, both of which preserve bone quality. However, a very interesting and plausible issue is the effect of vitamin

D in combination with calcium in decreasing the risk of falls. Bischoff-Ferrari *et al.* (2004) conducted another meta-analysis study among 1,237 subjects as a collection of 5 randomized controlled trials. They found that the group that received vitamin D in combination with calcium decreased the risk of falling by 22% [corrected OR, 0.78, 95% CI 0.64-0.92] compared to the group that received only calcium or the placebo. This study showed the number needed to treat to be 15 [95% CI, 8-53] which is encouraging in terms of health economy. To conclude, vitamin D supplementation with the administration of calcium could effectively decrease the risk of falls in elderly people by more than 20% (Bischoff-Ferrari *et al.*, 2004).

However, this study did not answer questions about the appropriate dose of vitamin D and calcium, the appropriate frequency and the method of treatment as well as the appropriate length of treatment. However, Bischoff-Ferrari *et al.* (2004) recommended the dose of vitamin D at 700-800 IU/day and recommended that the accompanying calcium supplement should always be administered as well. Generally, we can see that the treatment of osteoporosis will focus on the baseline of patients, i.e. that they should receive adequate treatment with calcium and vitamin D. As mentioned before, even elderly people living in hot areas might still receive less than adequate amounts of vitamin D. Also, the general diet in Thailand mostly contains low levels of calcium, hence the treatment of osteoporosis in Thai elderly people should always put great emphasis on treatment with vitamin D and calcium supplement. In addition to antiresorptive treatments for the general indication, vitamin D and calcium supplements can at least help decrease the risk of falls which is an alternative mechanism for decreasing the risk of fracture, as well as enhancing the better anti-fracture efficacy of other medicines.

Reference

Atkins, G.J., Findlay, D.M., Anderson, P.H. and Morris, H.A., 2005. Target genes: bone proteins. In: Feldman, D., Pike, J.W. and Glorieux, R.H. (eds.) Vitamin D. 2nd ed., Elsevier Academic Press, San Diego, CA, USA, pp.711-720.

Binkley, N., Krueger, D., Cowgill, C.S., Plum, L., Lake, E., Hansen, K.E., DeLuca, H.F. and Drezner M.K., 2004. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. The Journal of Clinical Endocrinology and Metabolism 89, 3152-3157.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Willett, W.C., Staehelin, H.B., Bazemore, M.G., Zee, R.Y. and Wong, J.B., 2004. Effect of vitamin D on falls: a meta-analysis. The Journal of the American Medical Association 291, 1999-2006.

Bischoff-Ferrari, H.A., Stahelin, H.B., Dick, W., Akos, R., Knecht, M., Salis, C., Nebiker, M., Theiler, R., Pfeifer, M., Begerow, B., Lew, R.A. and Conzelmann, M., 2003. Effect of vitamin D and calcium supplementation on falls: a randomized controlled trial. Journal of Bone and Mineral Research 18, 343-351.

Bischoff-Ferrari, H.A., Stahelin, H.B., Urscheler. N., Ehrsam, R., Vonthelin, R., Perrig-Chiello, P., Tyndall, A. and Theiler, R., 1999. Muscle strength in elderly: its relation to vitamin D metabolites. Archives Physical Medicine Rehabilitation 80, 54-58.

Boland, R., 1986. Role of vitamin D in skeletal muscle function. Endocrine Review 7, 434-448.

Boland, R.L., 2005. Vitamin D and muscle. In: Feldman, D., Pike, J.W. and Glorieux, R.H. (eds.) Vitamin D. 2nd ed., Elsevier Academic Press, San Diego, CA, USA, pp. 883-898.

Boland, R.L., De Boland, A.R., Marinissen, M.J., Santillan, G., Vazquez, G. and Zanello, S.B., 1995. Avian muscle cells as targets for the seco-steroid hormone 1,25-dihydroxy-vitamin D3. Molecular and Cellular Endocrinology 114, 1-8.

Boland, R.L., Norman, A.W., Ritz, E. and Hasselbach, W., 1985. Presence of a 1,25-dihydroxy-vitamin D3 receptor in chick skeletal muscle myoblast. Biochemical Biophysical Research Communications 128, 305-311.

Brunner, A. and De Boland, A.R., 1990. 1,25-Dihydroxyvitamin D3 affects the synthesis, phosphorylation and in vitro calmodulin binding of myoblast cytoskeletal proteins. Zeitschrift fur Naturforschung 45, 1156-1160.

Chailurkit, L., Teerarungsikul, K., Rajatanavin, R., Ongphiphadhanakul, B. and Puavilai, G., 1996. Serum vitamin D, parathyroid hormone and biochemical markers of bone turnover in normal Thai subjects. Journal of the Medical Association of Thailand 79, 499-504.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P.D. and Meunier, P.J., 1992. Vitamin D3 and and calcium to prevent hip fractures in the elderly women. The New England Journal of Medicine 327, 1637-1642.

Chapuy, M.C., Preziosi, P., Maamer, M., Amaud, S., Galan, P., Hercberg, S. and Meunier, P.J., 1997. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis International 7, 439-443.

Dawson-Hughes, B., Harris, S., Krall, E.A. and Dallal, G.E., 1997b. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. The New England Journal of Medicine 337, 670-676.

Dawson-Hughes, B., Harris, S.S. and Dallal, G.E., 1997a. Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. The American Journal of Clinical Nutrition 65, 67-71.

Dawson-Hughes, B., Heaney, R.P., Holick, M.F., Lips, P., Meunier, P.J. and Vieth, R., 2005. Estimates of optimal vitamin D status. Osteoporosis International 16, 713-716.

De Boland, A. and Nemere, I., 1992. Rapid actions of vitamin D compounds. Journal of Cellular Biochemistry 49, 32-36.

De Boland, A.R., Morelli, S. and Boland, R., 1995. 1,25$(OH)_2$-vitamin D3 stimulates phospholipase A_2 activity via a guanine nucleotide-binding protein in chick myoblasts. Biochimica et Biophysica Acta 1257, 274-278.

De Boland, A.R., Morelli, S. and Boland, R.L., 1994. 1,25$(OH)_2$-vitamin D3 signal transduction in chick myoblast involves phosphatidycholine hydrolysis. Journal of Biological Chemistry 269, 8675-8679.

Demay, M., 2003. Muscle: a nontraditional 1,25-dihydroxyvitamin D target tissue exhibiting classic hormone-dependent vitamin D receptor actions. Endocrinology 144, 5135-

Dhesi, J.K., Moniz, C., Close, J.C., Jackson, S.H. and Allain, T.J., 2002. A rationale for vitamin D prescribing in falls clinic population. Age and Ageing 31, 267-271.

Drittanti, L., Boland, R. and De Boland, A.R., 1989a. Induction of specific proteins in cultured skeletal muscle cells by 1,25-dihydroxyvitamin D3. Biochimica et Biophysica Acta 1012, 16-23.

Drittanti, L., De Boland, A.R. and Boland, R., 1988. Effects of 1,25-dihydroxyvitamin D3 on phospholipid metabolism in chick myoblasts. Biochimica et Biophysica Acta 962, 1-7.

Drittanti, L., De Boland, A.R. and Boland, R., 1990. Stimulation of calmodulin synthesis in proliferating myoblasts by 1,25-dihydroxy-vitamin D3. Molecular and Cellular Endocrinology 74, 143-153.

Drittanti, L., De Boland, A.R. and Boland, R.L., 1989b. Modulation of DNA synthesis in cultured muscle cells by 1,25-dihydroxyvitamin D3. Biochimica et Biophysica Acta 1014, 112-119.

Ebashi, S. and Endo, M., 1968. Calcium ion and muscle contraction. Progress in Biophysics and Molecular Biology 18, 123-183.

Endo, I., Inoue, E., Mitsui, T., Umaki, Y., Akaike, M., Yoshizawa, T., Kato, S. and Matsumoto, T., 2003. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology 144, 5138-5144.

Feldman, D., Pike, J.W. and Glorieux, R.H. (eds), 2005. Vitamin D. 2nd ed. Elsevier Academic Press, San Diego, CA, USA.

Gannage-Yared, M.H., Chemali, R., Yaacoub, N. and Halaby, G., 2000. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. Journal of Bone and Mineral Research 15, 1856-1862.

Geusens, P., Vandevyver, C., Vanhoof, J., Cassiman, J.J., Boonen, S. and Raus, J., 1997. Quadriceps and grip strength are related to vitamin D receptor genotype in elderly non-obese women. Journal of Bone and Mineral Research 12, 2082-2088.

Ghannam, N.N., Hammami, M.M., Bakheet, S.M. and Khan, B.A., 1999. Bone mineral density of the spine and femur in healthy Saudi female: relation to vitamin D status, pregnancy, and lactation. Calcified Tissue International 65, 23-28.

Glerup, H. and Eriksen, E.F., 2005. Muscles and falls. In: Feldman, D., Pike, J.W. and Glorieux, R.H. (eds.) Vitamin D. 2nd ed., Elsevier Academic Press, San Diego, CA, USA, pp.1805-1820.

Glerup, H., 1999. Investigations on the role of vitamin D in muscle function: a study of muscle function in vitamin D deficient humans and the effect of treatment with vitamin D. Ph.D.Thesis,, University of Aarhus, Denmark.

Glerup, H., Mikkelsen, K., Poulsen, L., Hass, E., Overbeck, S., Andersen, H., Charles, P. and Eriksen, E.F., 2000. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. Calcified Tissue International 66, 419-424.

Goswami, R., Gupta, N., Goswami, D., Marwaha, R.K., Tandon, N. and Kochupillai, N., 2000. Prevalence and significant of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. The American Journal of Clinical Nutrition 72, 472-475.

Graafmans, W.C., Ooms, M.E., Hofstee, H.M.A., Bezemer, P.D., Bouter, L.M. and Lips, P., 1996. Falls in the elderly: a prospective study of risk factors and risk profiles. American Journal of Epidemiology 143, 129-136.

Hanley, D.A., Cranney, A., Jones, G., Whiting, S.J. and Leslie, W.D., 2010. for the Guidelines Committee of the Scientific Advisory Council of Osteoporosis Canada. Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada. Canadian Medical Association Journal 182, 1315-1319.

Heaney, R.P., 2003a. Human serum 25-hydroxy-cholecalciferol response to extended oral dosing with cholecalciferol. The American Journal of Clinical Nutrition 77, 204-210.

Heaney, R.P., 2003b. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. Journal of the American College of Nutrition 22, 142-146.

Heaney, R.P., 2003c. Long-latency deficiency disease: insights from calcium and vitamin D. The American Journal of Clinical Nutrition 78, 912-919.

Heaney, R.P., 2005. The vitamin D requirement in health and disease. The Journal of Steroid Biochemistry and Molecular Biology 97, 13-19.

Holick, M.F., 2000. Calcium and vitamin D. diagnostics and therapeutics. Clinics in Laboratory Medicine 20, 569-590.

Holick, M.F., 2003. Vitamin D deficiency: what a pain it is. Mayo Clinic Proceedings 78, 1457-1459.

Holick, M.F., 2007. Vitamin D deficiency. The New England Journal of Medicine 357, 266-281.

Holick, M.F., 2008. Photobiology and noncalcemic actions of vitamin D. In: Bilezikian, J.P., Raisz, L.G. and Martin, T.J. (eds.) Principle of Bone Biology. 3rd ed., Elsevier Academic Press, San Diego, CA, USA, pp.795-812.

Holick, M.F., Matsuoka, L.Y. and Wortsman, J., 1989. Age, vitamin D, and solar ultraviolet radiation. The Lancet 334, 1104-1105.

Holick, M.F., Schnoes, H.K., DeLuca, H.F., Suda, T. and Cousins, R.J., 1971. Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. Biochemistry 10, 2799-2804.

Kinyamu, H.K., Gallagher, J.C., Rafferty, K.A. and Balhorn, K.E., 1998. Dietary calcium and vitamin D intake in elderly women: effect on serum parathyroid hormone and vitamin D metabolites. The American Journal of Clinical Nutrition 67, 342-348.

Lawson, D.E., Wilson, P.W., Kodicek, F., Morrison, H.R. and Williams, D.H., 1971. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. Nature 230, 228-230.

Li, Y.C., Amling, M., Pirro, A.E., Priemel, M., Meuse, J., Baron, R., Delling, G. and Demay, M.B., 1998. Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor ablated mice. Endocrinology 139, 4391-4396.

Malabanan, M., 1998. Redefining vitamin D insufficiency. The Lancet 351, 805-806.

McComas, A.J., 1996. Skeletal muscle: form and function. Human Kinetics, Champaign, pp.62-65.

McDonnell, D.P., Mangelsdorf, D.J., Pike, J.W., Haussler, M.R. and O'Malley, B.W., 1987. Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. Science 235, 1214-1217.

Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhardt, P., Dawson-Hughes, B., Eisman, J.A., El-Hajj Fuleihan, G., Josse, R.G., Lips, P. and Morales-Torres, J., 2009. on behalf of the IOF Committee of Scientific Advisors (CSA) Nutrition Working Group. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International 20, 1807-1820.

Morelli, S., Boland, R. and De Boland, A.R., 1996. 1,25$(OH)_2$-vitamin D3 Stimulation of phospholipases C and D in muscle cells involves extracellular calcium and a pertussive-sensitive G protein. Molecular and Cellular Endocrinology 122, 207-211.

Morelli, S., De Boland, A.R. and Boland, R.L., 1993. Generation of inositol phosphates, diacylglycerol and calcium fluxes in myoblasts treated with 1,25-dihydroxyvitamin D3. Biochemical Journal 289, 675-679.

Mowe, M., Haug, E. and Bohmer, T., 1999. Low serum calcidiol concentration in older adults with reduced muscular function. Journal of the American Geriatric Society 47, 220-226.

National Osteoporosis Foundation (NOF), 2008. Universal recommendations for all patients. In: 2008 Clinician's Guide to Prevention and Treatment of Osteoporosis. NOF, Washington DC, US, pp.16-18.

Nemere, I. and Farach-Carson, M.C., 1998. Membrane receptors for steroid hormones: a case for specific cell surface binding sites for vitamin D metabolites and estrogens. Biochemical Biophysical Research Communications 248, 443-449.

Norman, A.W., 1968. The mode of action of vitamin D. Biological Reviews 43, 97-135.

Norman, A.W., Myrtle, J.F., Midgett, R.J., Nowicki, H.G., Williams, V. and Popjak, G., 1971. 1,25-Dihydroxy-cholecalciferol: identification of the proposed active form of vitamin D in intestine. Science 173, 51-54.

Pike, J.W. and Shevde, N.K., 2005. The vitamin D receptor. In: Feldman, D., Pike, J.W. and Glorieux, R.H. (eds.) Vitamin D. 2nd ed., Elsevier Academic Press, San Diego, CA, USA, pp. 167-191.

Sato, Y., Iwamoto, J., Kanoko, T. and Satoh, K., 2005. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. Cerebrovascular Diseases 20, 187-192.

Skaria, J., Katiyar, B.C., Srivastava, T.P. and Dube, B., 1975. Myopathy and Neuropathy associated with osteomalacia. Acta Neurologica Scandinavica 51:37-58.

Songpatanasilp, T., Chailurkit, L., Nichachotsalid, A. and Chantarasorn, M., 2009. Combination of alfacalcidol with calcium can improve quadriceps muscle strength in elderly ambulatory Thai women who have hypovitaminosis D: a randomized controlled trial. Journal of the Medical Association of Thailand 92(Suppl5), S30-41.

Soontrapa, S., Soontrapa, S., Pongchaiyakul, C., Somboonporn, C., Somboonporn, W. and Chailurkit, L., 2001. Prevalence of hypovitaminosis D in elderly women living in urban area of Khon Kaen province, Thailand. Journal of the Medical Association of Thailand 84(suppl 2), S534-S541.

Sorensen, O.H., Lund, B., Saltin, B., Lund, B., Andersen, R.B., Hjorth, L., Melsen, F. and Mosekilde, L., 1979. Myopathy in bone loss of ageing: improvement by treatment with 1 alpha-hydroxycholecalciferol and calcium. Clinical Science 56, 157-161.

Stein, M.S., Wark, J.D., Scherer, S.C., Walton, S.L., Chick. P., Di Carlantonio, M., Zajac, J.D. and Flicker, L., 1999. Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel. Journal of the American Geriatric Society 47, 1195-1201.

Steingrimsdottir, L., Gunnarsson, O., Indridason, O.S., Franzson, L. and Sigurdsson, G., 2005. Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. The Journal of the American Medical Association 294, 2336-2341.

Sutton, A.L. and McDonald, P.N., 2003. Vitamin D: more than a 'bone-a-fide' hormone. Molecular Endocrinology 17, 777-791.

Tinetti, M.E., Speechley, M. and Ginter, S.F., 1988. Risk for falls among elderly persons living in the community. The New England Journal of Medicine 319, 1701-1707.

Vasquez, A., 2004. Integrative orthopaedics: concepts, algorithms, and therapeutics. Natural Health Consulting Corporation, Houston, US, pp. 417-419.

Vieth, R., 1999. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. The American Journal of Clinical Nutrition 69, 842-856.

Walters, M., 1992. Newly identified functions of the vitamin D endocrine system. Endocrine Review 13, 719-764.

Yoshikawa, S., Nakamura, T., Tanabe, H. and Imamura, T., 1979. Osteomalacic myopathy. Endocrinologia Japonica 26, 65-72.

Zanello, S.B., Boland, R.L. and Norman, A.W., 1995. cDNA sequence identity of a vitamin D-dependent calcium-binding protein in the chick to calbindin D-9K. Endocrinology 136, 2784-2787.

Zittermann, A., 2003. Vitamin D in preventive medicine: are we ignoring the evidence? British Journal of Nutrition 89, 552-557.

Key facts

- Vitamin D deficiency is highly prevalent among children and adolescents due to limited sunlight exposure and inadequate dietary intake.
- Vitamin D is required for a normal function of the body.
- Altogether 37 tissues present vitamin D receptor (VDR)
- Deficiency affects growth and development, but if corrected while growing most of the consequences are overcome.
- VDR polymorphism may modify the response of vitamin D supplementation.

Summary points

- Vitamin D deficiency causes rickets affecting the well-being of an individual in multiple ways, i.e. skeletal mineralization, axial growth, immune responses and muscle function.
- Maternal vitamin D status is associated with bone size and bone mass in newborn in several studies, and this association may remain throughout childhood.
- In severely vitamin D deficient infants even small dosages of vitamin D appear to improve growth.
- High doses are needed to overcome rickets and its skeletal consequences.
- Vitamin D supplementation benefits bone mineral accrual in apparently healthy prepubertal girls. Most pronounced effects are seen on cortical rich sites.
- Although VDR polymorphism may explain differences observed in growth pattern, stature or bone mineral density, it is more likely to modify the response to vitamin D supplementation.

7. Vitamin D and growth

H.T. Viljakainen[1,2] and E. Hyppönen[3]
[1]Children's Hospital, University of Helsinki, P.O. Box 705, 00029 HUS, Finland; [2]Musculoskeletal Research Unit, School of Clinical Sciences, University of Bristol, Southmead Hospital, BS10 5NB Bristol, United Kingdom; [3]Centre for Paediatric Epidemiology and Biostatistics and Medical Research Council Centre for the Epidemiology of Child Health, University College London Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom;
heli.viljakainen@helsinki.fi

Abstract

Linear growth requires health and adequate nutrition. Stunted growth is a growth disorder with multiple symptoms. In Asia 35-65% of under 5-year olds have stunted growth. Rickets is a consequence of severe vitamin D deficiency and can be seen as a special case for stunted growth. Nutritional vitamin D status is commonly measured with serum 25-hydroxyvitamin D (25(OH)D). Clinical symptoms of rickets are typically associated to 25(OH)D level below 12.5 nmol/l, but the normal function of body may require concentrations above 50 nmol/l. Rickets may relate to delayed or difficulties with crawling and walking and it commonly presents with comorbidities such as continuous or recurrent respiratory infections. We reviewed studies reporting both bone mass accrual and linear growth as an outcome and vitamin D as an exposure. 'Vitamin D' includes here intake of vitamin D, it's nutritional status, and closely related genetic variations. We further evaluate studies using vitamin D to prevent stunting of growth, and also characterize the effect of vitamin D deficiency induced rickets on growth. Vitamin D deficiency causes rickets affecting the well-being of an individual in multiple ways, i.e. skeletal mineralization, axial growth, immune responses and muscle function. Maternal vitamin D status is associated with bone size and bone mass in newborn in several studies, and this association may remain throughout childhood. In severely vitamin D deficient infants even small dosages of vitamin D appear to improve growth, but to overcome rickets and its skeletal consequences high doses are needed. Vitamin D intervention benefits the bone mineral accrual in apparently healthy prepubertal girls. Most pronounced effects are seen on cortical rich sites and to lesser extent in trabecular bone. Although vitamin D receptor polymorphism may explain differences observed in growth pattern, stature or BMD, it is more likely to modify the response to vitamin D supplementation.

Keywords: growth, skeletal outcomes, children, adolescent, long-term consequences, VDR polymorphism, supplementation

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_7, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
BMC	Bone mineral content
BMD	Bone mineral density
CSA	Cross-sectional area
FGF-23	Fibroblast growth factor 23
IGF-1	Insulin-like growth factor 1
RCT	Randomized controlled trial
SGA	Small for gestational age
UVB	Ultraviolet B
VDR	Vitamin D receptor

7.1 Introduction

Linear growth requires health and adequate nutrition. Careful monitoring of growth helps to reveal underlying disease in an individual. At the population level, growth patterns reflect general well-being and changes in living standards over decades. A pragmatic way to describe growth is to measure increases in height, weight or circumference (head or mid-upper arm) over time. During childhood, growth and development are tightly linked, and it is often difficult distinguish influences on linear growth from those affecting the process of maturation.

Stunted growth is a growth disorder with multiple symptoms. It is very common in low income countries and is thought to be a complex implication of nutritional, environmental and social factors. According to a Unicef report (Unicef, 2012) as many as 35-65% of under 5-year olds have stunted growth in the region of India and China. This is illustrated by lower than -2 SD (or less than 10^{th} percentile) average birth weight in these populations, ceased growth rate and eventually small size in adulthood. Improved standards of living associate with lower rate of stunted infant growth. Because of its complexity interventions to prevent stunted growth are hard to establish. Rickets is a well-known consequence of severe vitamin D deficiency and can be seen as a special case for stunted growth. Nutritional vitamin D status is commonly measured by circulating concentrations of 25(OH)D. Clinical symptoms of rickets are typically associated to 25(OH)D level below 12.5 nmol/l (always below 25 nmol/l) (Bishop, 1999). While there is no general consensus for adequate vitamin D concentrations, recent review by Institute of Medicine indicated that concentrations over 50 nmol/l are required for normal function of body (Institute of Medicine, 2011). In this chapter, we will review studies reporting influences both on bone mass accrual and linear growth with the exposure 'vitamin D' including the intake of vitamin D, it's nutritional status, and closely related genetic variations. We will further evaluate studies using vitamin D to prevent stunting of growth, and also characterize the effect of vitamin D deficiency induced rickets on growth.

7.2 General about growth and bone mass accrual

During fetal life increase in bone mass occurs mainly through intramembranous ossification. Highest rate of calcium accretion is noted during the third trimester (Prentice *et al.*, 2006) together with highest growth velocity (Gardosi, 1997). Growth velocity continues to be high immediately after birth, slows down after 2 years and accelerates again later in childhood, finally ceasing after puberty. Typically longitudinal bone growth in infancy affects more appendicular than axial skeleton, which matures in puberty (Boot *et al.*, 1997). Growth in the length of the long bones occurs in the growth plates through endochondral ossification; a cartilage rich form of bone matures and calcifies into bone (Heaney *et al.*, 2000). Longitudinal bone growth ceases after puberty as the growth plates fuse, but modeling in term of mineral apposition continues throughout the life (Heaney *et al.*, 2000). Periosteal apposition increases the outer diameter of bone: mineral is placed far away from central axis to increase mechanical strength. Androgens and weigh bearing exercise stimulate periosteal apposition, which explains why men and athletes have bigger bones. Opposite to this is modifications of endosteal surface, thus a phenomenon responsible for wall thickness. In females extra mineral is placed under endosteal envelope, where it can be utilized for increased requirements later in life (Prentice *et al.*, 2006).

Growth trajectory is suggested to be set during the fetal period: metabolic and endocrine systems are programmed to adapt the fetus to the outside-utero environment (Jansson and Powell, 2007). This may explain seasonal variation of birth outcomes, e.g. birth weight, overall size, limb length, head size and skin-fold thickness observed in several studies (McGrath *et al.*, 2005; Murray *et al.*, 2000). Influences on fetal growth may be mediated through growth hormone – IGF-I axis, whereas bone quality may be determined by factors related to differentiation of mesenchymal stem cells (Lanham *et al.*, 2008; Zhou *et al.*, 2010). The intrauterine environment is suggested to have long-term consequences on growth up until puberty: intrauterine nutritional deficits have permanent consequences and that a newborn's metabolism may not adapt to improved nutritional status (Gluckman and Hanson, 2004). To support this proposition, several studies have reported a relationship between growth pattern (during the fetal period, infancy and early childhood) and lower adult BMD or increased fracture risk later in life (Cooper *et al.*, 1997, 2001; Yarbrough *et al.*, 2000).

7.3 Rickets and growth

Vitamin D deficiency in childhood is the most common cause of rickets, which is characterized by disturbed bone formation: bone formation rate is accelerated but mineralization of growth plates is stunted (Parfitt, 2003). This affects the shaping and the strength of bones, leading to low BMD and bowing of weight-bearing bones (Bishop, 1999). Metaphyseal bone sites are typically widened in patients with rickets. Rickets may relate to delayed or difficulties with crawling and walking (Agarwal *et al.*, 2009; Thacher *et al.*, 2000), and it commonly presents with comorbidities such as continuous or recurrent respiratory infections (Karatekin *et al.*, 2009; Laaksi *et al.*, 2007; Wayse *et al.*, 2004) and muscle weakness (Pettifor and Prentice, 2011). Osteomalasia is the adult form of rickets, and results from a chronic vitamin D deficiency affecting both bone

and muscle. It is more difficult to diagnose because of a mixture of related symptoms which include muscle or bone pain, increased risk of fractures, and progressive deformities (Narchi *et al.*, 2001). Neonatal consequences of vitamin D deficiency include hypocalcemia, big fontanelles, and bone deformities. Severe cases can present with seizures with extreme complications such as cardiac failure reported in some studies (Maiya *et al.*, 2008; Uysal *et al.*, 1999). Asymptomatic, mild rickets may remain unrecognized during growth, but related consequences can sometimes present later in life with symptoms such as increases in the risk of fractures or complications with labor due to related bone malformations in the pelvis.

Bone modeling and mineralization occurs in metaphysis of long bones whereas epiphysis is responsible for longitudinal growth (Rauch *et al.*, 2001). In rickets newly formed bone is hypomineralized, but the rate of longitudinal growth is normal or even accelerated (Parfitt, 2003). This means that in rickets the collagenous format of the bone remains longer and is readily deformed to bowlegs, asymmetrical skull or bulges in the ribcage (Pettifor and Prentice, 2011). Bone growth is illustrated by bone turnover markers: during growth both formation and resorption markers are increased. Growth is an anabolic situation and formation overrules bone turnover. Several studies have demonstrated that in rickets especially bone specific alkaline phosphatase and pro-collagen type I aminoterminal peptide are elevated because of activated osteoblasts but not osteocalcin (Brooke *et al.*, 1980; Soliman *et al.*, 2010). However, after correction of vitamin D deficiency, bone turnover typically decelerates and markers of mineralized matrix such as osteocalcin are normalized.

7.4 Maternal vitamin D status, fetal bone mineralization and growth

The effect of maternal vitamin D status on fetal bone mass has been a focus in several prospective studies (Akcakus *et al.*, 2006; Viljakainen *et al.*, 2010; Weiler *et al.*, 2005), but as reviewed below, findings have been somewhat inconsistent. In a Finnish study maternal vitamin D status was measured twice during pregnancy at 13th week and 2 days postpartum, the mean of these two was most strongly associated with newborn bone characteristics (Viljakainen *et al.*, 2010). The median S-25(OH)D in the group of 100 women was less than 50 nmol/l, with nearly 70% of mothers having concentration below 50 nmol/l. Children born to mothers with inferior vitamin D status had 16% smaller bone CSA and 14% lower mineral content in distal tibia compared to children born to mothers with superior vitamin D status (Viljakainen *et al.*, 2010). The bone measures were performed from the diaphysis of tibia with peripheral quantitative computed tomography. Larger bone size accompanied with higher mineral content accounts for higher bone strength at birth. In contrast, Akcakus *et al.* (2006) found no relationship between maternal vitamin D status and infant bone indices at birth. This could be due to extremely poor vitamin D status in the whole cohort, since nearly 90% of mother-child pairs had 25(OH)D below 25 nmol/l. In another study, lower maternal 25(OH)D was related to greater fetal femoral metaphyseal CSA, but not to femoral length at 19 and 34 weeks' gestation (Mahon *et al.*, 2010). Femoral characteristics were measured with high-resolution 3D ultrasound from over 400 mothers in Southampton in UK. Authors calculated spaying index (a ratio of CSA to length) which is used to characterize rickets and this was also associated to lower maternal 25(OH)D concentration.

Findings were somewhat controversial from a study on full-term and normal birth weight infants, where vitamin D deficiency was associated with greater weight and length but lower bone mass relative to body weight (Weiler *et al.*, 2005). This finding could be a consequence of faster growth *in utero* which intervenes maternal vitamin D status since corresponding has been reported (Viljakainen *et al.*, 2010). These findings could also suggest that mothers with larger babies had lower vitamin D status at the end of the gestation than mothers with smaller babies. Similarly, it is proposed that maternal parathyroid hormone concentrations increase due to greater mineral demands of bigger babies (Brooke *et al.*, 1980). Vitamin D status of pregnant women is typically lower compared to non-pregnant women (Holmes *et al.*, 2009), with concentrations declining as the pregnancy proceeds (Holmes *et al.*, 2009). Declining concentrations may be caused by volumetric dilution, or possibly, because of increased vitamin D requirement induced by the growing fetus.

There are only a few studies investigating the association between maternal vitamin D status and birth length, while more studies have focused on birth weight. In an Australian study, newborn to vitamin D deficient mothers had 4.5 mm shorter knee-heel length; thought to be most reliable measure of intrauterine long bone growth (Morley *et al.*, 2006). Newborn to vitamin D deficient mothers also had smaller mid-upper arm and calf circumference than newborn to mothers with superior vitamin D status. In this study, vitamin D deficiency was also associated with shorter duration of gestation by 0.7 week, which appeared to explain differences in knee-heel length at least partly, and no differences in circumferences were noted after adjusting for gestation length.

There are conflicting reports on maternal intake of vitamin D and offspring birth weight (Scholl and Chen, 2009). In a study from Teheran correlations were found between adequate maternal calcium and vitamin D intake with both appropriate birth weight and weight gain of mothers during pregnancy (Sabour *et al.*, 2006). A large-scale prospective study in Netherlands searched for an association between maternal vitamin D status with fetal and neonatal growth in a multi-ethnic cohort (n=3,730) (Leffelaar *et al.*, 2010). They compared newborn size and growth in three groups which were defined by maternal vitamin D status (≤29.9, 30-49.9, ≥50 nmol/l) at 12th week of gestation. Mothers and their newborns were followed 12 months after the birth. Altogether 2,355 children completed the study with detailed growth records. Compared to group with adequate vitamin D status (≥50 nmol/l) children born to mothers with inferior 25(OH)D had lower birth weight (3,418, 3,506 and 3,560 g, in above mentioned groups, respectively) and higher proportion of them were considered as SGA (14.8, 9.4 and 6.8%, in groups, respectively). However, ethnicity was not equally distributed across groups, which complicates the interpretation of results. Crude values showed that difference in birth weight between deficient and adequate group was 64 (29-107.5) grams in favor of adequate vitamin D status and similarly the risk for SGA was 1.9 times higher (1.4-2.7) after adjusting for possible confounders including ethnicity (Leffelaar *et al.*, 2010).

Experimental evidence for an association with maternal vitamin D intake and infant growth from supplementation trials with vitamin D is scarce, but results are relatively consistent. One of the earliest randomized controlled trials studied Asian originated immigrants in United

Kingdom. Participants in the study, although a part of an affluent society in south London, were severely vitamin D deficient (mean 20 nmol/l) at enrolment which was between 28 and 32 weeks' gestation (Brooke *et al.*, 1980). Daily supplementation with 1000 IU improved maternal and cord 25(OH)D concentration and maintained normalized serum calcium and total alkaline phosphate activity in cord blood when compared to placebo group. However, no differences in mean weight, length, or other anthropometrics were observed at birth. On the other hand, 50% lower proportion of newborns in the supplemented group were SGA when compared to placebo (Brooke *et al.*, 1980). These findings were supported with a similarly study design (Maxwell *et al.*, 1981). In addition, they observed that maternal weight gain was greater in supplemented group. Brooke's group extended the follow up of the mother and child pairs (Brooke *et al.*, 1981). After 1 year of follow up they reported that children whose mothers received supplementation during pregnancy continued to grow more efficiently and achieved higher weight and length at the age of 12 months (Brooke *et al.*, 1981). Recently, Hollis and Wagner (2013) performed randomized trial on pregnant women with daily dose of 400 IU, 2,000 IU or 4,000 IU throughout the pregnancy. They showed that delivery mode and co-morbidies such as preeclampsia differed between groups in favor of higher supplementation. A tendency for longer gestational age (P=0.1) with increasing supplementation was also noted, but no difference in mean birth weight. Shorter gestational length induced by poor maternal vitamin D status could potentially explain some of the differences in birth size as discussed above.

7.5 Maternal vitamin D status, bone density and growth in childhood

There are some studies to suggest that maternal vitamin D status has long-term consequences on bone growth and mineralization in the offspring. A prospective study of Javaid *et al.* showed in 198 subjects that maternal vitamin D status during late pregnancy predicts total body and lumbar spine BMC and BMD at age 9 (Javaid *et al.*, 2006). In the same cohort, higher maternal vitamin D status was associated also with greater head circumference, but there were no consistent associations with growth, intelligence, or psychological health (Gale *et al.*, 2008). However, some caution is required when interpreting these data, as only 38% of the original cohort attended the 9-year visit.

Based on correlations with estimated maternal UVB exposure and bone outcomes of offspring, Sayers and Tobias suggested maternal vitamin D status to affect skeletal development in child directly through influences on periosteal bone formation (Sayers and Tobias, 2009). Their study was done in Avon Longitudinal Study of Parents and Children birth cohort consisting of nearly 7,000 subjects (Sayers and Tobias, 2009), and they reported associations between estimated maternal UVB exposure in late pregnancy with offspring bone mass and size, height, and lean mass at 10 years. At the time of the study, information on maternal 25(OH)D concentrations was available only for a sub-sample of 55 mothers (mean 53 (SD=32) nmol/l), nevertheless, a weak positive correlation with estimated UVB dose was reported. Although the maternal vitamin D status appears crucial for bone growth, the prospective study from Finland (Viljakainen *et al.*,

2011) suggests that catch-up growth occurs in bone mineral accrual, but not in bone size, when vitamin D status is corrected in initially deficient infants.

7.6 Infant vitamin D supplementation, bone density and growth in childhood

A retrospective study of Zamora *et al.* (1999) tracked the effect of infant vitamin D supplementation (dosage 400 IU) on bone health in pre-pubertal Swiss girls. Children who had taken vitamin D supplements in infancy had higher areal BMD in radial metaphysis, femoral neck, and trochanter, but not in trabecular rich bone sites, e.g. lumbar spine. Unfortunately, authors did not analyze supplementation in relation to changes in childhood height or weight gain. However, this study design might be biased due to low number of subjects in the control group (n=15) and no data on current use of vitamin D supplementation.

The effect of vitamin D supplementation on infant growth has been a neglected topic, but a high quality study was recently published in British Medical Journal. In this RCT the effect of weekly supplementation of vitamin D on mortality and morbidity in Indian low birth weight, full term infants was investigated (Kumar *et al.*, 2011). Secondary outcome in this trial was growth at the age of 6 months. Stunted infant growth is typical in India and it was one of the outcomes in the study. Despite adequate power calculations, mortality or morbidity were not affected by vitamin D supplementation, but increases in length, weight, and arm circumference were significantly higher in supplemented group compared to placebo at 6-months. Authors emphasized that impact on anthropometrics were relatively small as difference between groups were about 0.11 Z-scores in favor of supplementation (Kumar *et al.*, 2011). Vitamin D dosage which was used in the study was relatively modest (1,400 IU/35 μg per week) and although it improved the mean 25(OH)D concentration by 19 nmol/l, 43% of children remained vitamin D deficient (25(OH)D <25 nmol/l) in the supplemented group. Nevertheless, these results are promising by suggesting clear benefits with correction of extreme vitamin D deficiency, but studies with a higher dose and longer follow-up time will help to clarify the results for anthropometric outcomes. In addition to vitamin D deficiency Indian infants in the study of Kumar *et al.* might suffer from multiple nutritional deficiencies (Kumar *et al.*, 2011). This is supported by a study of Ekbote and colleagues (2011) in which 47% were severely vitamin D deficient, 70% had anemia, roughly half presented stunted growth, and possibly also protein malnutrition at baseline. Laddoon, a traditional Indian sweet was tested as a vehicle for calcium supplementation to 60 Indian toddlers aged between 2 and 3 years. In addition to calcium fortified laddoon which was provided five days a week both groups received once a month an oral dose of vitamin D 30,000 IU. The intervention did not affect growth *per se*, but children in a group receiving higher calcium fortification gained more mineral to their skeleton and presented higher BMD at the end of the study compared to the control group.

In contrast to the proposed benefits for vitamin D supplementation on growth outlined above, a high dose vitamin D supplementation has also been suggested to be harmful for linear growth. This suggestion has been largely based on relatively old and small case studies on children, in

particular one study from 1937 which reported data for 9 cases suggestive of potential reductions in growth (Jeans and Stearns, 1938). However, using data from a large prospective population-based birth cohort of nearly 10,000 children, Hyppönen and colleagues did not find any evidence for stunted growth for infants who had received high dose vitamin D supplementation (typically 2,000 IU/day) during the first year of life (Hypponen *et al.*, 2011); indeed before confounder adjustment supplemented children were significantly taller than others. However, it was not possible to exclude a possible adverse association between high-dose vitamin D supplementation and growth in height in infants who are born small at birth based on this study, and a possibility also remains that a co-supplementation with vitamin A may have reduced any possible adverse effects by high dose vitamin D supplementation(Hypponen *et al.*, 2011). Interestingly, rickets at 1 year of age was not associated with length of the child in this study. However, infants who had been affected by rickets during the first year of life tended to be relatively tall in adulthood, and once the authors accounted for rickets treatment and adult height (as a proxy for growth potential) rickets became expectedly associated with shorter length. This could be due to children who became tall adults being more susceptible to rickets possible due to higher requirements for growth (Hypponen *et al.*, 2011).

7.7 Vitamin D supplementation and childhood growth: intervention studies

A sizeable amount of peak bone mass accrues during puberty and many researchers have focused on nutrition and bone mass accrual during this critical period. After birth, vitamin D has a role in bone development, particularly with bone mineralization (El-Hajj Fuleihan *et al.*, 2006; Lehtonen-Veromaa *et al.*, 2002b; Viljakainen *et al.*, 2006). It has also been suggested that effect of vitamin D on bone may act through muscle (Bartoszewska *et al.*, 2010), but the current evidence on the association of muscle and vitamin D is conflicting (El-Hajj Fuleihan *et al.*, 2006; Ward *et al.*, 2010) and a direct effect of vitamin D on bone cells via regulation of the calcium balance is more commonly accepted (Norman, 2008).

There are several studies investigating the effect of varying dosages and types of vitamin D supplementation on bone mineralization and growth, with largely inconsistent findings. In an early study by Ala-Houhala *et al.* (1988), 54 girls aged between 8 and 10 years were randomized to receive either vitamin D (400 IU/day) or placebo for 12 months (Ala-Houhala *et al.*, 1988). The study was underpowered to detect differences between groups either in height increment or bone mineral accretion in distal radius. More recently, another Finnish group investigated the effect of wintertime vitamin D2 supplementation on bone mineral accrual in initially 9-15 year old girls during a 3-year trial (Lehtonen-Veromaa *et al.*, 2002b). They found that the baseline S-25(OH)D predicted the 3-year change in lumbar BMD, however, they failed to show any effects on bone mineral accrual by supplementation with vitamin D (10 or 20 μg/day). The higher dose given during wintertime raised S-25(OH)D concentration to 44 nmol/l, which was significantly higher than in the placebo group (32 nmol/l) (Lehtonen-Veromaa *et al.*, 2002a).

Also in the 24-month trial of Cheng *et al.* (2005) vitamin D supplementation with calcium to girls with habitually low calcium diet did not improve bone mineral accrual in whole body or hip and did not increase tibian strength compared to placebo. However, in their study calcium supplementation in terms of high consumption of cheese appeared to increase cortical thickness tibia more when compared to other groups.

In contrast to earlier studies with smaller samples, we identified four RCTs with positive effects. A dose-response effect of vitamin D3 on bone mineral accrual both in total hip and lumbar spine area was shown in prepubertal Finnish girls (n=228) with high habitual calcium intake (Viljakainen *et al.*, 2006). In this study, the participants were first stratified by their pubertal status and then randomized into three study groups. The intervention overlapped with a period of high growth rate. Interestingly, girls in the vitamin D groups gained more weight as well as mineral to their bones than in the placebo (Viljakainen *et al.*, 2006). After adjusting for changes in weight, bone size and pubertal status; BMC accretion in the hip was 14% and 17% higher in the groups receiving 5 μg (200 IU) and 10 μg (400 IU) of vitamin D, respectively, compared with the placebo group. Only the higher dose increased lumbar spine BMC significantly. Also a Canadian study with vitamin D 10 μg and calcium a day showed an association with BMC and BMD, while the intervention did not affect tibian length. In this study the subjects were Caucasian girls (n=77), aged 12 years, and they had an early pubertal status (Moyer-Mileur *et al.*, 2003). Furthermore, in the recent study by Mølgaard *et al.* (2010) the effect of vitamin D supplementation on bone mineral accrual was modified by VDR genotype. This study on apparently healthy 11-year old Danish girls, did not find any overall differences in whole-body or lumbar spine size-adjusted BMC after a 12 month vitamin D supplementation (5-10 μg day) when compared to placebo. However, the group reported an interaction with supplementation and *Fok*I VDR gene polymorphisms in whole-body BMD: vitamin D supplementation improved BMC over placebo in a sub-group of girls who carried the FF VDR phenotype (Mølgaard *et al.*, 2010). Further studies are required to explore gene-environment interaction in affecting response to vitamin D supplementation, as this may explain why some individuals are more responsive to vitamin D supplementation than others.

A Lebanese study reported increments in lean mass, bone size, and BMC in hip, but not in lumbar spine, or total body (El-Hajj Fuleihan *et al.*, 2006). Altogether 179 girls were randomly assigned to receive either weekly dose of 14,000 IU, 1,400 IU, or placebo for 12 months. The increments in bone mass appeared largest at the highest dose and especially in the subgroup of premenarcheal girls. Interestingly, this study also reported a weak tendency for enhanced height increment across vitamin D supplemented groups (5.6%, 5.0%, 3.8%, in groups 14,000 IU, 1,400 IU, or placebo, respectively (*P=0.07*)).

Milk interventions may provide wider understanding on impact of nutrients on growth and bone development. Depending on the study design milk may be fortified with extra calcium and vitamin D. However, in different study populations the effects of the trials vary (Cheng *et al.*, 2005; Zhu *et al.*, 2006). Differences in response may be explained with differences in general nutritional status, growth pattern, and activity levels. A 2-year school milk intervention in China

consisted of three arms (Zhu *et al.*, 2006): no milk, 330 ml milk fortified with Ca, and 330 ml milk fortified with both Ca and vitamin D. Two of the latter ones provided extra calcium and vitamin D 560 mg, 0 μg and 560 mg, 5-8 μg, respectively. After the 2-year intervention both fortified groups showed grater increase in height (0.7%), sitting height (1%) and BMC (1.5%) and BMD (4.3%) compared to placebo group (Zhu *et al.*, 2006). In the beginning of the study Chinese girls aged 10 years and were mostly prepubertal.

Soliman *et al.* (2010) observed that Nigerian children suffering from severe vitamin D deficiency were short for age, had lower BMI and their growth velocity was decreased compared to apparently healthy controls. The children were treated with mega doses of vitamin D (10,000 IU/kg) intramuscularly. After 6 months children were still shorter, but their other antropometric measurements had improved to comparable level with controls with a significant increase in growth velocity. One of the most intriguing observations the group made was that S-25(OH)D concentration was associated with circulating IGF-1 level (Soliman *et al.*, 2010), a finding that has also been seen in adults (Hypponen *et al.*, 2008). It has been proposed that regain in growth velocity after the correction of vitamin D deficiency is mediated through growth hormone/IGF-I axis (Soliman *et al.*, 2010).

The short-term benefit of vitamin D supplementation on bone mass accrual is at least promising, but in a long follow up the effects tend to attenuate. On the other hand, calcium supplementation is suggested to extend growth period (Chevalley *et al.*, 2005b; Rozen *et al.*, 2003) which implies the plasticity of growing phenotype is maintained in optimal environment. It is speculated that intervention with vitamin D supplementation during puberty might have persistent effects (Chevalley *et al.*, 2005a), but this is not supported by all studies (Zhu *et al.*, 2006).

7.8 Vitamin D and growth: animal models

There is some evidence for the importance of vitamin D on growth from animal studies and in particular, features of vitamin D dependent rickets in domestic animals appear to be very similar to those observed in humans (Dittmer and Thompson, 2011). Most frequently rickets was observed in sheep or pigs with scarce or no UVB exposure accompanied with poor, unbalanced diet. The musculoskeletal symptoms in pigs were similar to humans: difficulty in rising, bowed forelegs, joint swellings, and pain on moving. Although sheep in many countries graze outside throughout the year, the covering fleece reduces exposure to ultraviolet light. Similarly to observations in humans, vitamin D deficiency in sheep is exacerbated during pregnancy and S-25(OH)D concentration of lambs and dam are closely related. Rickets was observed most commonly in rapidly growing lambs.

In contrast, rodents do not typically become totally vitamin D deficient as discussed (Hollis and Wagner, 2013) and there is evidence to suggest that vitamin D may not be required for fetal growth or development because the influences of calcitriol on calcium absorption are compensated by other hormones (Fudge and Kovacs, 2010). It is possible, that in rodents during

weaning these compensatory mechanisms may maintain normal skeletal mineralization in the offspring (Fudge and Kovacs, 2010), however, it is unclear if such influences may be applicable to humans. Differences in the length of pregnancy and placental structure may also contribute to the extent by which studies on maternal vitamin D status and fetal development done in rodents will apply to humans.

Despite differences between the mice and men, the phenotype of genetically modified animals such VDR- or 1-α-hydroxylase null mice can provide valuable information on the possible role of vitamin D for well-being. A recent review discussed these in detail in comparison with findings in man (Bouillon *et al.*, 2008). At birth fetuses of VDR null mice were slightly growth-retarded and their skeleton undermineralized: total Ca content per weight and the mineralized bone in the CSA of tibia were reduced (Rummens *et al.*, 2003). On the contrary, growth plate was normal. A rescue diet with high calcium, phosphorus and lactose recovered the metabolic and skeletal abnormalities of VDR null mice, but in the fetuses, the rescue diet reversed only the bone phenotype but not the growth retardation. Interestingly, VDR null fetuses on the rescue diet gained less weight than on the standard diet, hence, the VDR genotype seems to determine fetal growth in mice as well as in humans as reviewed below. However, VDR null mice appear to grow similarly to wild-type mice while and after weaning (Li *et al.*, 1997). After weaning weight gain was more slow in homozygous VDR knockout mice compared to wild-type and heterozygous VDR knockout mice (Figure 7.1), and VDR ablated animals presented impaired muscle function together with mixed neurological symptoms (Endo *et al.*, 2003).

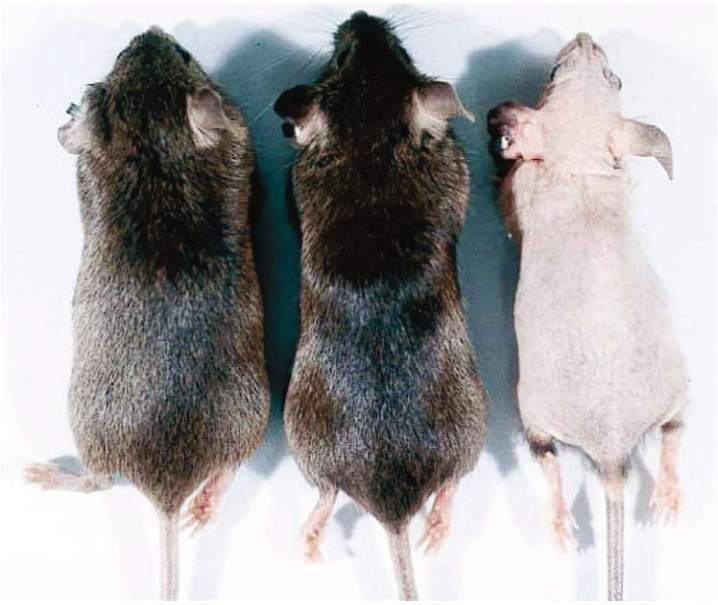

Figure 7.1. Phenotype of VDR knock-out mice at 3.5 months of age. The genotypes of the mice, from left to right, are wild type, $VDR^{-/+}$ and $VDR^{-/-}$. Adapted from Li *et al.* (1997).

7.9 Genetic studies in humans: association with growth and bone outcomes

Gene CYP2R1 codes liver enzyme primarily responsible for the hydroxylation of vitamin D. Mutation in CYP2R1 is rare, but it will have profound effects on vitamin D metabolism such as impaired clearance of vitamin D, and severe rickets with multiple endocrine defects (Cheng *et al.*, 2004). Although there is another mitochondrial enzyme capable of hydroxylating vitamin D it is unable to cover the actions of CYP2R1. A recent case report on a family with an autosomal dominant CYP2R1 mutation confirmed that lack of function mutation of CYP2R1 affects growth and predisposes to severe rickets and will results to short stature at adulthood if not treated (Tosson and Rose, 2012).

The function of vitamin D in the body depends on how efficiently it is bound to a specific carrier, vitamin D binding protein, also called GC protein. Interestingly genetic variation in GC is associated with 25(OH)D bioavailability to target cells (Chun *et al.*, 2010). However, in humans GC protein deficiency is not known, but genetic variation in GC might affect metabolic rate and clinical outcomes. In clinical studies GC protein phenotype is associated with circulating 25(OH)D levels and fracture risk (Lauridsen *et al.*, 2004, 2005). Because genetic variation in GC goes together with skin color, this might explain why vitamin D deficiency presents differently in different populations (Berry and Hypponen, 2011), but its relevance on growth or bone mass accrual remains to be discovered.

VDR is recognized as a candidate gene associated with bone outcomes and growth because of its functional relevance, plausible minor allele frequency and its relative position in choromosome 12 is described with sequence variation (Van der Sluis *et al.*, 2003). Frequent polymorphisms in the VDR gene appear to contribute to growth *in utero* and in early infancy (Bodnar *et al.*, 2010; Keen *et al.*, 1997; Lorentzon *et al.*, 2000; Morley *et al.*, 2009; Suarez *et al.*, 1997), and notably adult height (Fang *et al.*, 2007; Lorentzon *et al.*, 2000; Macgregor *et al.*, 2008). Morley and colleagues observed that low birth weight newborn to vitamin D deficient mothers carried more frequently FF or Ff VDR phenotype (Morley *et al.*, 2009). VDR gene polymorphism was significantly associated with risk of SGA in a recent study (Bodnar *et al.*, 2010), but this differed by race. Furthermore, they gained evidence that VDR genotype and 25(OH)D concentration may have independent contributions to risk of SGA (Bodnar *et al.*, 2010). A Swedish study reported that length at birth, increase in height from birth to early adulthood, stature at 19 years as well as bone size varied by VDR Bsm polymorphisms (Lorentzon *et al.*, 2000). Also in a study of obese children BsmI was associated with differences in average height, with the effect being most pronounced in postpubertal children (Ferrarezi *et al.*, 2012). Association was independent of 25(OH)D or insulin sensitivity, which in some other studies has been related to VDR polymorphism (Jain *et al.*, 2012; Oh and Barrett-Connor, 2002). A recent meta-analysis with over 14,000 subjects suggested that carriers of Bsm 'BB' genotype were approximately 0.6 cm (CI: 0.2-1.1 cm) taller than those with the 'bb' genotype; the effect of allelic variant was independent of age, sex or age related height loss (Fang *et al.*, 2007). These findings were partly supported by McGregor *et al.* (2008), but they concluded that the effect size explains approximately 0.1% of the phenotypic variance in height, thus probably hundreds of such variants are responsible for the observed

genetic variation in height. VDR polymorphisms have also been related to BMD in some (Arabi *et al.*, 2009; Laaksonen *et al.*, 2004; Strandberg *et al.*, 2003; Viitanen *et al.*, 1996) but not in all studies (Gunnes *et al.*, 1997; Lei *et al.*, 2005), and the results tend to vary according to gender, and age. Higher stature together with grater lumbar spine width, but no difference in BMD in lumbar spine or tibia were observed in carriers of particular haplotype (created by three adjacent restriction enzymes, 'bAT') in Caucasian children and young adults in Netherlands (Van der Sluis *et al.*, 2003). Correspondingly, increase in BMC in response to vitamin D supplementation was in one study observed to be dependent on VDR Bsm genotype (Arabi *et al.*, 2009). Because bone gain was affected by genotype only in supplemented groups authors suggested that VDR polymorphism does not affect bone mass accrual per se, but modifies the response to vitamin D supplementation (Arabi *et al.*, 2009).

7.10 Insights in to mechanisms of action

Calcitriol is an endocrine hormone and known to influence bone mineral accrual by contributing to calcium homeostasis. Calcium is the major constituent of skeleton, but its actions (absoption, resorption and excretion) in the body are regulated by vitamin D either directly or indirectly (Norman, 2008). However, the action of calcitriol in body may be quite complex as illustrated by Norman (2008) (Figure 7.2). Furthermore, a feed-back loop through FGF-23 is identified. After mineralization of collagen matrix, mature osteocytes produce FGF-23 to down-regulate 1-α-hydroxylation of 25(OH)D (Bouillon and Decallonne, 2010). Besides, in bone vitamin D up-regulates osteocalcin production which is identified as an endocrine regulator of glucose metabolism. Secondly, paracrine action of vitamin D exists. Vitamin D affects cell cycle and cell differentiation; whether a mesenchymal stem cell differentiates to osteoblast, muscle cell, neuron, or adipocyte depends on availability of 25(OH)D. At the moment there are about 40 target organs for vitamin D. This means that 25(OH)D can enter these cells and transform to calcitriol locally and influence gene expression of selected genes. Zhou *et al.* (2010) demonstrated in human bone marrow stromal cells, that vitamin D metabolites (25(OH)D) upregulate the production of IGF-1 which allows the differentiation of stromal cells into osteoblasts. Similarly, IGF-1 was found to promote periosteal growth and bone mass accrual indirectly through muscle growth in a seven year long follow-up (Xu *et al.*, 2011). In addition, studies on cell cultures have proved that IGF-1 is produced locally by osteoblasts and muscle cells under the regulation of vitamin D (Gurlek *et al.*, 2002). Regardless of the cell type the functions of vitamin D in different target cells are anabolic.

In addition, genetic variation may involve lower copy numbers of VDR (in carriers of risk haplotypes) which could explain differences observed in stature or BMD, but more likely it seems that response to vitamin D supplementation is more distinctive according to genotype (Arabi *et al.*, 2009; Bodnar *et al.*, 2010).

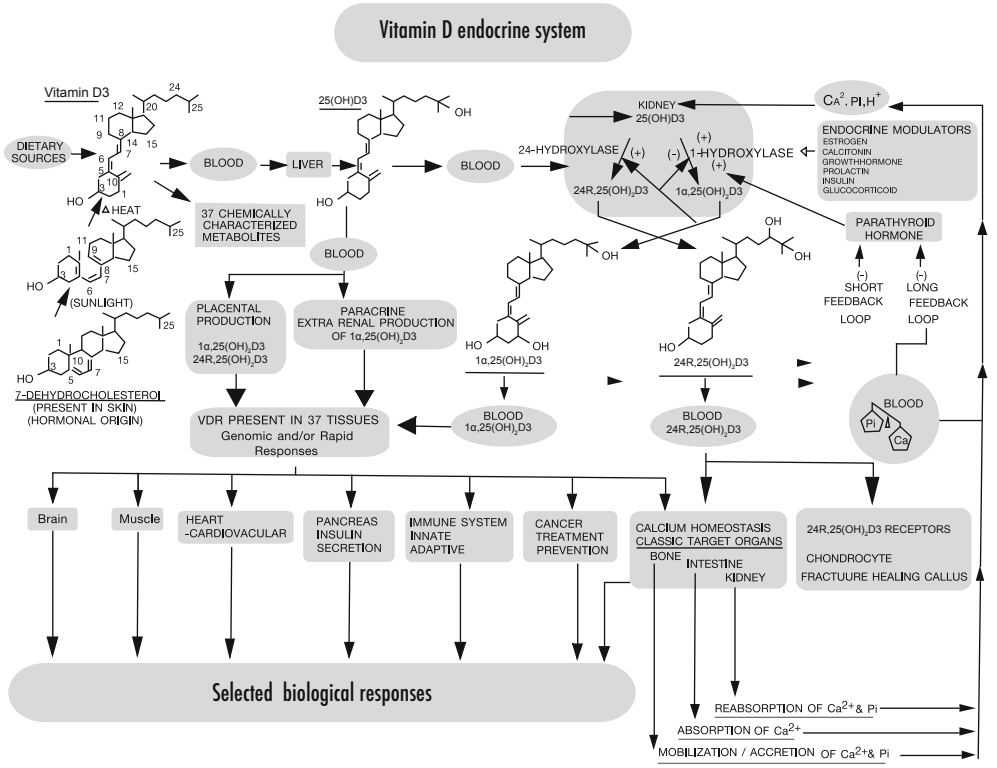

Figure 7.2 Overview of vitamin D endocrine system (adapted from Norman, 2008).

7.11 Summary

Although there are only few high quality RCTs on vitamin D and childhood bone outcomes, evidence is broadly supportive of a beneficial effect, with further enforcements coming from observational studies and animal models. The most pronounced effects appear to be seen in prepubertal girls with high habitual calcium intake and on cortical rich sites as total hip or whole body and to lesser extent in lumbar spine. There is an amounting body of evidence suggesting that maternal vitamin D status is positively related to bone size and bone mass at birth, and this association may remain throughout childhood. However, the association between higher intake of vitamin D and higher axial growth needs to be confirmed in further studies. Little is also known on potential threshold effects, and it is possible that the positive associations between vitamin D and bone mass/density are most pronounced in initially deficient populations. Correspondingly the long-term effects of vitamin D supplementation on bone outcomes will need to be confirmed in future studies.

Evidence on the effects of vitamin D on linear growth is surprisingly limited and relies largely on observational studies which are unable to prove the causality. Some effects may be indirect, for example vitamin D deficient children are likely to be more prone to illnesses such as acute

respiratory infections (Karatekin *et al.*, 2009; Wayse *et al.*, 2004) and some chronic diseases (Hollams, 2012; Hypponen, 2010; Hypponen *et al.*, 2001) which through influences on general well-being of the child may affect growth (Wayse *et al.*, 2004). In severely vitamin D deficient infants even small dosages of vitamin D appear to improve growth (Kumar *et al.*, 2011). However, more studies are needed to establish the effect of vitamin D supplementation on linear growth during childhood, and whether benefits will be seen beyond with the effects on the correction of severe vitamin D deficiency.

References

Agarwal, A., Gulati, D., Rath, S. and Walia, M., 2009. Rickets: a cause of delayed walking in toddlers. Indian Journal of Pediatrics 76, 269-272.

Akcakus, M., Koklu, E., Kurtoglu, S., Kula, M. and Koklu, S.S., 2006. The relationship among intrauterine growth, insulinlike growth factor I (IGF-I), IGF-binding protein-3, and bone mineral status in newborn infants. American Journal of Perinatology 23, 473-480.

Ala-Houhala, M., Koskinen, T., Koskinen, M. and Visakorpi, J.K., 1988. Double blind study on the need for vitamin D supplementation in prepubertal children. Acta Paediatrica Scandinavica 77, 89-93.

Arabi, A., Zahed, L., Mahfoud, Z., El-Onsi, L., Nabulsi, M., Maalouf, J. and Fuleihan Gel, H., 2009. Vitamin D receptor gene polymorphisms modulate the skeletal response to vitamin D supplementation in healthy girls. Bone 45, 1091-1097.

Bartoszewska, M., Kamboj, M. and Patel, D.R., 2010. Vitamin D, muscle function, and exercise performance. Pediatric Clinics of North America 57, 849-861.

Berry, D. and Hypponen, E., 2011. Determinants of vitamin D status: focus on genetic variations. Current Opinion in Nephrology and Hypertension 20, 331-336.

Bishop, N., 1999. Rickets today – children still need milk and sunshine. New England Journal of Medicine 341, 602-604.

Bodnar, L.M., Catov, J.M., Zmuda, J.M., Cooper, M.E., Parrott, M.S., Roberts, J.M., Marazita, M.L. and Simhan, H.N., 2010. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. Journal of Nutrition 140, 999-1006.

Boot, A.M., de Ridder, M.A., Pols, H.A., Krenning, E.P. and de Muinck Keizer-Schrama, S.M., 1997. Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. Journal of Clinical Endocrinology and Metabolism 82, 57-62.

Bouillon, R., Bischoff-Ferrari, H. and Willett, W., 2008. Vitamin D and health: perspectives from mice and man. Journal of Bone and Mineral Research 23, 974-979.

Bouillon, R. and Decallonne, B., 2010. The white adipose tissue connection with calcium and bone homeostasis. Journal of Bone and Mineral Research 25, 1707-1710.

Brooke, O.G., Brown, I.R., Bone, C.D., Carter, N.D., Cleeve, H.J., Maxwell, J.D., Robinson, V.P. and Winder, S.M., 1980. Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. British Medical Journal 280, 751-754.

Brooke, O.G., Butters, F. and Wood, C., 1981. Intrauterine vitamin D nutrition and postnatal growth in Asian infants. British Medical Journal (Clinical Research Ed.) 283, 1024.

Cheng, J.B., Levine, M.A., Bell, N.H., Mangelsdorf, D.J. and Russell, D.W., 2004. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci U S A 101, 7711-7715.

Cheng, S., Lyytikainen, A., Kroger, H., Lamberg-Allardt, C., Alen, M., Koistinen, A., Wang, Q.J., Suuriniemi, M., Suominen, H., Mahonen, A., Nicholson, P.H., Ivaska, K.K., Korpela, R., Ohlsson, C., Vaananen, K.H. and Tylavsky, F., 2005. Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10-12-y-old girls: a 2-y randomized trial. American Journal of Clinical Nutrition 82, 1115-1126; quiz 1147-1118.

Chevalley, T., Bonjour, J.P., Ferrari, S., Hans, D. and Rizzoli, R., 2005a. Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: a randomized, double-blind, placebo-controlled trial in prepubertal boys. Journal of Clinical Endocrinology and Metabolism 90, 3342-3349.

Chevalley, T., Rizzoli, R., Hans, D., Ferrari, S. and Bonjour, J.P., 2005b. Interaction between calcium intake and menarcheal age on bone mass gain: an eight-year follow-up study from prepuberty to postmenarche. Journal of Clinical Endocrinology and Metabolism 90, 44-51.

Chun, R.F., Lauridsen, A.L., Suon, L., Zella, L.A., Pike, J.W., Modlin, R.L., Martineau, A.R., Wilkinson, R.J., Adams, J. and Hewison, M., 2010. Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. Journal of Clinical Endocrinology and Metabolism 95, 3368-3376.

Cooper, C., Eriksson, J.G., Forsen, T., Osmond, C., Tuomilehto, J. and Barker, D.J., 2001. Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. Osteoporosis International 12, 623-629.

Cooper, C., Fall, C., Egger, P., Hobbs, R., Eastell, R. and Barker, D., 1997. Growth in infancy and bone mass in later life. Annals of the Rheumatic Diseases 56, 17-21.

Dittmer, K.E. and Thompson, K.G., 2011. Vitamin D metabolism and rickets in domestic animals: a review. Veterinary Pathology 48, 389-407.

Ekbote, V.H., Khadilkar, A.V., Chiplonkar, S.A., Hanumante, N.M., Khadilkar, V.V. and Mughal, M.Z., 2011. A pilot randomized controlled trial of oral calcium and vitamin D supplementation using fortified laddoos in underprivileged Indian toddlers. European Journal of Clinical Nutrition 65, 440-446.

El-Hajj Fuleihan, G., Nabulsi, M., Tamim, H., Maalouf, J., Salamoun, M., Khalife, H., Choucair, M., Arabi, A. and Vieth, R., 2006. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. Journal of Clinical Endocrinology and Metabolism 91, 405-412.

Endo, I., Inoue, D., Mitsui, T., Umaki, Y., Akaike, M., Yoshizawa, T., Kato, S. and Matsumoto, T., 2003. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology 144, 5138-5144.

Fang, Y., van Meurs, J.B., Rivadeneira, F., van Schoor, N.M., van Leeuwen, J.P., Lips, P., Pols, H.A. and Uitterlinden, A.G., 2007. Vitamin D receptor gene haplotype is associated with body height and bone size. Journal of Clinical Endocrinology and Metabolism 92, 1491-1501.

Ferrarezi, D.A., Bellili-Munoz, N., Nicolau, C., Cheurfa, N., Guazzelli, I.C., Frazzatto, E., Velho, G. and Villares, S.M., 2012. Allelic variations in the vitamin D receptor gene, insulin secretion and parents' heights are independently associated with height in obese children and adolescents. Metabolism 61, 1413-1421.

Fudge, N.J. and Kovacs, C.S., 2010. Pregnancy up-regulates intestinal calcium absorption and skeletal mineralization independently of the vitamin D receptor. Endocrinology 151, 886-895.

Gale, C.R., Robinson, S.M., Harvey, N.C., Javaid, M.K., Jiang, B., Martyn, C.N., Godfrey, K.M. and Cooper, C., 2008. Maternal vitamin D status during pregnancy and child outcomes. European Journal of Clinical Nutrition 62, 68-77.

Gardosi, J., 1997. Customized growth curves. Clinical Obstetrics and Gynecology 40, 715-722.

Gluckman, P.D. and Hanson, M.A., 2004. Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. Pediatric Research 56, 311-317.

Gunnes, M., Berg, J.P., Halse, J. and Lehmann, E.H., 1997. Lack of relationship between vitamin D receptor genotype and forearm bone gain in healthy children, adolescents, and young adults. Journal of Clinical Endocrinology and Metabolism 82, 851-855.

Gurlek, A., Pittelkow, M.R. and Kumar, R., 2002. Modulation of growth factor/cytokine synthesis and signaling by 1alpha,25-dihydroxyvitamin D(3): implications in cell growth and differentiation. Endocrine Reviews 23, 763-786.

Heaney, R.P., Abrams, S., Dawson-Hughes, B., Looker, A., Marcus, R., Matkovic, V. and Weaver, C., 2000. Peak bone mass. Osteoporosis International 11, 985-1009.

Hollams, E.M., 2012. Vitamin D and atopy and asthma phenotypes in children. Current Opinion in Allergy and Clinical Immunology 12, 228-234.

Hollis, B.W. and Wagner, C.L., 2013. Vitamin D and pregnancy: skeletal effects, nonskeletal effects, and birth outcomes. Calcified Tissue International 92, 128-139.

Holmes, V.A., Barnes, M.S., Alexander, H.D., McFaul, P. and Wallace, J.M., 2009. Vitamin D deficiency and insufficiency in pregnant women: a longitudinal study. British Journal of Nutrition 102, 876-881.

Hypponen, E., 2010. Vitamin D and increasing incidence of type 1 diabetes-evidence for an association? Diabetes Obes Metab 12, 737-743.

Hypponen, E., Boucher, B.J., Berry, D.J. and Power, C., 2008. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. Diabetes 57, 298-305.

Hypponen, E., Fararouei, M., Sovio, U., Hartikainen, A.L., Pouta, A., Robertson, C., Whittaker, J.C. and Jarvelin, M.R., 2011. High-dose vitamin D supplements are not associated with linear growth in a large Finnish cohort. Journal of Nutrition 141, 843-848.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R. and Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358, 1500-1503.

Institute of Medicine, F.a.N.B., 2011. Dietary Reference Intakes for Calcium and Vitamin D, Institute of Medicine, Washington, D.C.

Jain, R., von Hurst, P.R., Stonehouse, W., Love, D.R., Higgins, C.M. and Coad, J., 2012. Association of vitamin D receptor gene polymorphisms with insulin resistance and response to vitamin D. Metabolism 61, 293-301.

Jansson, T. and Powell, T.L., 2007. Role of the placenta in fetal programming: underlying mechanisms and potential interventional approaches. Clinical Science (London, England: 1979) 113, 1-13.

Javaid, M.K., Crozier, S.R., Harvey, N.C., Gale, C.R., Dennison, E.M., Boucher, B.J., Arden, N.K., Godfrey, K.M. and Cooper, C., 2006. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 367, 36-43.

Jeans, P.C. and Stearns, G., 1938. The effect of vitamin D on linear growth in infancy II The effect of intakes above 1,800 USPunits daily. Journal of Pediatrics 13, 730-740.

Karatekin, G., Kaya, A., Salihoglu, O., Balci, H. and Nuhoglu, A., 2009. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. European Journal of Clinical Nutrition 63, 473-477.

Keen, R.W., Egger, P., Fall, C., Major, P.J., Lanchbury, J.S., Spector, T.D. and Cooper, C., 1997. Polymorphisms of the vitamin D receptor, infant growth, and adult bone mass. Calcified Tissue International 60, 233-235.

Kumar, G.T., Sachdev, H.S., Chellani, H., Rehman, A.M., Singh, V., Arora, H. and Filteau, S., 2011. Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. BMJ 342, d2975.

Laaksi, I., Ruohola, J.P., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamaki, H. and Ylikomi, T., 2007. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. American Journal of Clinical Nutrition 86, 714-717.

Laaksonen, M.M., Karkkainen, M.U., Outila, T.A., Rita, H.J. and Lamberg-Allardt, C.J., 2004. Vitamin D receptor gene start codon polymorphism (FokI) is associated with forearm bone mineral density and calcaneal ultrasound in Finnish adolescent boys but not in girls. Journal of Bone and Mineral Metabolism 22, 479-485.

Lanham, S.A., Roberts, C., Cooper, C. and Oreffo, R.O., 2008. Intrauterine programming of bone. Part 1: alteration of the osteogenic environment. Osteoporosis International 19, 147-156.

Lauridsen, A.L., Vestergaard, P., Hermann, A.P., Brot, C., Heickendorff, L., Mosekilde, L. and Nexo, E., 2005. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcified Tissue International 77, 15-22.

Lauridsen, A.L., Vestergaard, P., Hermann, A.P., Moller, H.J., Mosekilde, L. and Nexo, E., 2004. Female premenopausal fracture risk is associated with gc phenotype. Journal of Bone and Mineral Research 19, 875-881.

Leffelaar, E.R., Vrijkotte, T.G. and van Eijsden, M., 2010. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. British Journal of Nutrition 104, 108-117.

Lehtonen-Veromaa, M., Mottonen, T., Nuotio, I., Irjala, K. and Viikari, J., 2002a. The effect of conventional vitamin D(2) supplementation on serum 25(OH)D concentration is weak among peripubertal Finnish girls: a 3-y prospective study. European Journal of Clinical Nutrition 56, 431-437.

Lehtonen-Veromaa, M.K., Mottonen, T.T., Nuotio, I.O., Irjala, K.M., Leino, A.E. and Viikari, J.S., 2002b. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. American Journal of Clinical Nutrition 76, 1446-1453.

Lei, S.F., Wang, Y.B., Liu, M.Y., Mo, X.Y. and Deng, H.W., 2005. The VDR, COL1A1, PTH, and PTHR1 gene polymorphisms are not associated with bone size and height in Chinese nuclear families. Journal of Bone and Mineral Metabolism 23, 501-505.

Li, Y.C., Pirro, A.E., Amling, M., Delling, G., Baron, R., Bronson, R. and Demay, M.B., 1997. Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. Proc Natl Acad Sci U S A 94, 9831-9835.

Lorentzon, M., Lorentzon, R. and Nordstrom, P., 2000. Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy caucasian men: a cross-sectional and longitudinal study. Journal of Clinical Endocrinology and Metabolism 85, 1666-1670.

Macgregor, S., Hottenga, J.J., Lind, P.A., Suchiman, H.E., Willemsen, G., Slagboom, P.E., Montgomery, G.W., Martin, N.G., Visscher, P.M. and Boomsma, D.I., 2008. Vitamin D receptor gene polymorphisms have negligible effect on human height. Twin Res Hum Genet 11, 488-494.

Mahon, P., Harvey, N., Crozier, S., Inskip, H., Robinson, S., Arden, N., Swaminathan, R., Cooper, C. and Godfrey, K., 2010. Low maternal vitamin D status and fetal bone development: cohort study. Journal of Bone and Mineral Research 25, 14-19.

Maiya, S., Sullivan, I., Allgrove, J., Yates, R., Malone, M., Brain, C., Archer, N., Mok, Q., Daubeney, P., Tulloh, R. and Burch, M., 2008. Hypocalcaemia and vitamin D deficiency: an important, but preventable, cause of life-threatening infant heart failure. Heart 94, 581-584.

Maxwell, J.D., Ang, L., Brooke, O.G. and Brown, I.R., 1981. Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. British Journal of Obstetrics and Gynaecology 88, 987-991.

McGrath, J.J., Keeping, D., Saha, S., Chant, D.C., Lieberman, D.E. and O'Callaghan, M.J., 2005. Seasonal fluctuations in birth weight and neonatal limb length; does prenatal vitamin D influence neonatal size and shape? Early Human Development 81, 609-618.

Molgaard, C., Larnkjaer, A., Cashman, K.D., Lamberg-Allardt, C., Jakobsen, J. and Michaelsen, K.F., 2010. Does vitamin D supplementation of healthy Danish Caucasian girls affect bone turnover and bone mineralization? Bone 46, 432-439.

Morley, R., Carlin, J.B., Pasco, J.A. and Wark, J.D., 2006. Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size. Journal of Clinical Endocrinology and Metabolism 91, 906-912.

Morley, R., Carlin, J.B., Pasco, J.A., Wark, J.D. and Ponsonby, A.L., 2009. Maternal 25-hydroxyvitamin D concentration and offspring birth size: effect modification by infant VDR genotype. European Journal of Clinical Nutrition 63, 802-804.

Moyer-Mileur, L.J., Xie, B., Ball, S.D. and Pratt, T., 2003. Bone mass and density response to a 12-month trial of calcium and vitamin D supplement in preadolescent girls. Journal of Musculoskeletal & Neuronal Interactions 3, 63-70.

Murray, L.J., O'Reilly, D.P., Betts, N., Patterson, C.C., Davey Smith, G. and Evans, A.E., 2000. Season and outdoor ambient temperature: effects on birth weight. Obstetrics and Gynecology 96, 689-695.

Narchi, H., El Jamil, M. and Kulaylat, N., 2001. Symptomatic rickets in adolescence. Archives of Disease in Childhood 84, 501-503.

Norman, A.W., 2008. A vitamin D nutritional cornucopia: new insights concerning the serum 25-hydroxyvitamin D status of the US population. American Journal of Clinical Nutrition 88, 1455-1456.

Oh, J.Y. and Barrett-Connor, E., 2002. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: the Rancho Bernardo Study. Metabolism 51, 356-359.

Parfitt, A.M., 2003. Renal bone disease: a new conceptual framework for the interpretation of bone histomorphometry. Current Opinion in Nephrology and Hypertension 12, 387-403.

Pettifor, J.M. and Prentice, A., 2011. The role of vitamin D in paediatric bone health. Best Practice & Research: Clinical Endocrinology & Metabolism 25, 573-584.

Prentice, A., Schoenmakers, I., Laskey, M.A., de Bono, S., Ginty, F. and Goldberg, G.R., 2006. Nutrition and bone growth and development. Proceedings of the Nutrition Society 65, 348-360.

Rauch, F., Neu, C., Manz, F. and Schoenau, E., 2001. The development of metaphyseal cortex – implications for distal radius fractures during growth. Journal of Bone and Mineral Research 16, 1547-1555.

Rozen, G.S., Rennert, G., Dodiuk-Gad, R.P., Rennert, H.S., Ish-Shalom, N., Diab, G., Raz, B. and Ish-Shalom, S., 2003. Calcium supplementation provides an extended window of opportunity for bone mass accretion after menarche. American Journal of Clinical Nutrition 78, 993-998.

Rummens, K., van Cromphaut, S.J., Carmeliet, G., van Herck, E., van Bree, R., Stockmans, I., Bouillon, R. and Verhaeghe, J., 2003. Pregnancy in mice lacking the vitamin D receptor: normal maternal skeletal response, but fetal hypomineralization rescued by maternal calcium supplementation. Pediatric Research 54, 466-473.

Sabour, H., Hossein-Nezhad, A., Maghbooli, Z., Madani, F., Mir, E. and Larijani, B., 2006. Relationship between pregnancy outcomes and maternal vitamin D and calcium intake: A cross-sectional study. Gynecological Endocrinology 22, 585-589.

Sayers, A. and Tobias, J.H., 2009. Estimated maternal ultraviolet B exposure levels in pregnancy influence skeletal development of the child. Journal of Clinical Endocrinology and Metabolism 94, 765-771.

Scholl, T.O. and Chen, X., 2009. Vitamin D intake during pregnancy: association with maternal characteristics and infant birth weight. Early Human Development 85, 231-234.

Soliman, A.T., El-Dabbagh, M., Adel, A., Al Ali, M., Aziz Bedair, E.M. and Elalaily, R.K., 2010. Clinical responses to a mega-dose of vitamin D3 in infants and toddlers with vitamin D deficiency rickets. Journal of Tropical Pediatrics 56, 19-26.

Strandberg, S., Nordstrom, P., Lorentzon, R. and Lorentzon, M., 2003. Vitamin D receptor start codon polymorphism (FokI) is related to bone mineral density in healthy adolescent boys. Journal of Bone and Mineral Metabolism 21, 109-113.

Suarez, F., Zeghoud, F., Rossignol, C., Walrant, O. and Garabedian, M., 1997. Association between vitamin D receptor gene polymorphism and sex-dependent growth during the first two years of life. Journal of Clinical Endocrinology and Metabolism 82, 2966-2970.

Thacher, T.D., Fischer, P.R., Pettifor, J.M., Lawson, J.O., Isichei, C.O. and Chan, G.M., 2000. Case-control study of factors associated with nutritional rickets in Nigerian children. Journal of Pediatrics 137, 367-373.

Tosson, H. and Rose, S.R., 2012. Absence of Mutation in Coding Regions of CYP2R1 Gene in Apparent Autosomal Dominant Vitamin D 25-Hydroxylase Deficiency Rickets. Journal of Clinical Endocrinology and Metabolism 97, E796-801.

Unicef, 2012. Tracking Progress on Child and Maternal Nutrition. In: Unicef (ed.), http://www.unicef.org/rightsite/sowc/pdfs/statistics/SOWC_Spec_Ed_CRC_TABLE%202.%20NUTRITION_EN_111309.pdf.

Uysal, S., Kalayci, A.G. and Baysal, K., 1999. Cardiac functions in children with vitamin D deficiency rickets. Pediatric Cardiology 20, 283-286.

Van der Sluis, I.M., De Muinck Keizer-Schrama, S.M., Krenning, E.P., Pols, H.A. and Uitterlinden, A.G., 2003. Vitamin D receptor gene polymorphism predicts height and bone size, rather than bone density in children and young adults. Calcified Tissue International 73, 332-338.

Viitanen, A., Karkkainen, M., Laitinen, K., Lamberg-Allardt, C., Kainulainen, K., Rasanen, L., Viikari, J., Valimaki, M.J. and Kontula, K., 1996. Common polymorphism of the vitamin D receptor gene is associated with variation of peak bone mass in young finns. Calcified Tissue International 59, 231-234.

Viljakainen, H.T., Korhonen, T., Hytinantti, T., Laitinen, E.K., Andersson, S., Makitie, O. and Lamberg-Allardt, C., 2011. Maternal vitamin D status affects bone growth in early childhood – a prospective cohort study. Osteoporosis International 22, 883-891.

Viljakainen, H.T., Natri, A.M., Karkkainen, M., Huttunen, M.M., Palssa, A., Jakobsen, J., Cashman, K.D., Molgaard, C. and Lamberg-Allardt, C., 2006. A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. Journal of Bone and Mineral Research 21, 836-844.

Viljakainen, H.T., Saarnio, E., Hytinantti, T., Miettinen, M., Surcel, H., Makitie, O., Andersson, S., Laitinen, K. and Lamberg-Allardt, C., 2010. Maternal vitamin D status determines bone variables in the newborn. Journal of Clinical Endocrinology and Metabolism 95, 1749-1757.

Ward, K.A., Das, G., Roberts, S.A., Berry, J.L., Adams, J.E., Rawer, R. and Mughal, M.Z., 2010. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. Journal of Clinical Endocrinology and Metabolism 95, 4643-4651.

Wayse, V., Yousafzai, A., Mogale, K. and Filteau, S., 2004. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. European Journal of Clinical Nutrition 58, 563-567.

Weiler, H., Fitzpatrick-Wong, S., Veitch, R., Kovacs, H., Schellenberg, J., McCloy, U. and Yuen, C.K., 2005. Vitamin D deficiency and whole-body and femur bone mass relative to weight in healthy newborns. CMAJ: Canadian Medical Association Journal 172, 757-761.

Xu, L., Wang, Q., Lyytikainen, A., Mikkola, T., Volgyi, E., Cheng, S., Wiklund, P., Munukka, E., Nicholson, P. and Alen, M., 2011. Concerted actions of insulin-like growth factor 1, testosterone, and estradiol on peripubertal bone growth: a 7-year longitudinal study. Journal of Bone and Mineral Research 26, 2204-2211.

Yarbrough, D.E., Barrett-Connor, E. and Morton, D.J., 2000. Birth weight as a predictor of adult bone mass in postmenopausal women: the Rancho Bernardo Study. Osteoporosis International 11, 626-630.

Zamora, S.A., Rizzoli, R., Belli, D.C., Slosman, D.O. and Bonjour, J.P., 1999. Vitamin D supplementation during infancy is associated with higher bone mineral mass in prepubertal girls. Journal of Clinical Endocrinology and Metabolism 84, 4541-4544.

Zhou, S., LeBoff, M.S. and Glowacki, J., 2010. Vitamin D metabolism and action in human bone marrow stromal cells. Endocrinology 151, 14-22.

Zhu, K., Zhang, Q., Foo, L.H., Trube, A., Ma, G., Hu, X., Du, X., Cowell, C.T., Fraser, D.R. and Greenfield, H., 2006. Growth, bone mass, and vitamin D status of Chinese adolescent girls 3 y after withdrawal of milk supplementation. American Journal of Clinical Nutrition 83, 714-721.

Key facts

- Rickets, a disease causing soft bones and severe growth retardation in children, was rampant in the industrialized cities of Europe and North America.
- Elmer McCollum at Johns Hopkins University, discovered Vitamin D in 1922 from cod liver oil as a dietary substance that could prevent rickets.
- In the last decade, there has been a resurgence of rickets in the developed world, leading to renewed interest in vitamin D.
- We now recognize that vitamin D has several non-calcemic biological functions and its impact on several chronic diseases, including arthritis, is an area of expanding research.

Summary points

- Vitamin D deficiency is a global epidemic.
- The major source of vitamin D synthesis in humans is cutaneous exposure to the sunlight.
- Vitamin D is a steroid hormone and exerts its diverse biological effects through the vitamin D receptor.
- Vitamin D is an immunomodulator and its role is initiation and exacerbation of autoimmune diseases is an area of ongoing research.
- Epidemiologic data indicate higher prevalence of autoimmune diseases in northern compared to southern latitudes, including inflammatory arthritis.
- Preliminary cross-sectional data suggest increased disease activity in rheumatoid arthritis patients with low vitamin D levels – these findings are not replicated in all studies.
- Low vitamin D levels may increase the risk of incident and progressive osteoarthritis – other studies do not confirm these findings.
- Direct causative role of vitamin D deficiency in arthritis is unclear.
- We await prospective long term interventional trials evaluating the role of vitamin D therapy in arthritis.

8. Vitamin D and arthritis

U.J. Haque
Johns Hopkins School of Medicine, Division of Rheumatology, 5200 Eastern Avenue Mason F. Lord Building, Center Tower, Suite 4100, Baltimore, MD 21224, USA; uhaque@jhmi.edu

Abstract

Vitamin D is a pleiotropic steroid molecule that exerts its physiological effects through the ubiquitous vitamin D receptor. Vitamin D is formed from its precursor in the skin upon exposure to the sunlight. Its importance in calcium homeostasis has been well known for a century, but recently several non-calcemic effects have been recognized. Vitamin D regulates several genes involved in cellular differentiation, apoptosis and has immunomodulatory effects. Epidemiologic data suggest that vitamin D deficiency may increase the risk of common cancers and autoimmune diseases. In experimental animal models, autoimmune diseases can be ameliorated by treatment with active vitamin D metabolites. Potential use of vitamin D supplementation to modify these diseases would certainly be a novel approach. Preliminary data from cross-sectional studies show that low vitamin D levels may be associated with increased disease activity in rheumatoid arthritis. Other studies show a possible increased risk of osteoarthritis with low vitamin D levels. These findings, while interesting, are not confirmed by all studies. Moreover, the cross-sectional design of these studies does not establish causality. Currently, the optimal and minimal level of vitamin D required for its physiological functions in humans is unknown. We, thus, await more research and randomized controlled interventional trials to answer these critical questions.

Keywords: steroid hormone, rheumatoid arthritis, osteoarthritis, disability, disease activity

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_8, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D3$	1,25-dihydroxyvitamin D3
25(OH)D	25-hydroxyvitamin D
BMI	Body mass index
CRP	C-reactive protein
DAS	Disease activity score
ESR	Erythrocyte sedimentation rate
MRI	Magnetic resonance imaging
MTX	Methotrexate
OA	Osteoarthritis
PBMC	Peripheral blood mononuclear cells
RA	Rheumatoid arthritis
TNF	Tumor necrosis factor
VDR	Vitamin D receptor

8.1 Introduction

Vitamin D is a pleiotropic steroid hormone and exerts its physiological effects through the ubiquitous VDR (Bikle, 2010). Activated vitamin D, via its interaction with VDR, is implicated in regulating several genes with diverse biological functions (Dong *et al.*, 2003; Sutton and MacDonald, 2003) which may explain its possible epidemiological association with several chronic diseases (Holick, 2007; Ponsonby *et al.*, 2002). The recent interest in vitamin D is driven by the recognition of its non-calcemic functions beyond its role in bone and calcium homeostasis. Vitamin D deficiency is endemic (Holick, 2007) and its potential impact on health is an area of expanding research. 25(OH)D, a circulating metabolite, can be measured in serum and is considered the best indicator of vitamin D status.

Vitamin D has known immunomodulatory effects (Maruotti and Cantatore, 2010) and its likely role in the initiation or exacerbation of autoimmunity is a topic of ongoing scientific study. In this chapter, we will explore potential association between vitamin D and arthritis. There are observational, epidemiological, experimental and clinical data that suggest a possible link between vitamin D and arthritis. However, the definite causative role of vitamin D deficiency in arthritis has not been established. We will review available data with particular reference to RA, the most common inflammatory arthritis characterized by erosive disease, increased disability and mortality. We will also briefly review the data regarding OA and vitamin D.

8.2 Vitamin D and rheumatoid arthritis

Although not found in all studies, one large observational study has shown increased risk of development of RA in women with lower vitamin D intake. In the Iowa Women's Health Study,

prospective follow up of 29,368 women revealed an inverse association between total vitamin D intake and the risk of RA (RR 0.67, 95% CI 0.44-1.00, *P*=0.05 for trend) (Merlino *et al.*, 2004). In this study, vitamin D levels were estimated using a self-administered dietary questionnaire only and 25(OH)D levels were not measured. This likely resulted in poor correlation with actual vitamin D status in this study and is its major limitation. Nielen *et al.* (2006) measured 25(OH)D levels in stored sera in a cohort of RA patients who had donated blood prior to onset of symptoms and compared these to matched healthy control. They did not observe any relationship between low levels of vitamin D and future risk of RA (Nielen *et al.*, 2006). Other studies also have observed no association between vitamin D intake and risk of RA (Costenbader *et al.*, 2008; Racovan *et al.*, 2011). In these studies, dietary vitamin D intake and solar irradiation were used to assess vitamin D status and actual 25(OH)D levels were not measured.

Epidemiological and observational evidence suggest a greater incidence of autoimmune diseases (Cantorna *et al.*, 2004; Ponsonby *et al.*, 2002), including inflammatory arthritis, with increasing latitude, similar to the amplified risk of cancer (Apperly and Cary, 1945; Grant, 2002). Higher prevalence of RA was noted in Northern vs. Southern Europe (Sokka and Pincus, 2005). Residents of northern climates are at a greater risk of vitamin D deficiency (Holick, 2007), as solar exposure of the skin is the main source of vitamin D synthesis in humans. Whether this solar gradient, via vitamin D deficiency, plays a causative role in conferring increased risk of arthritis, is unknown.

Data from animal models have also suggested a link between vitamin D and autoimmune diseases. In a study on collagen induced arthritis, mice fed a diet supplemented with $1,25(OH)_2D3$ had 50% less incidence of collagen induced arthritis compared to the control mice (Cantorna *et al.*, 1998). $1,25(OH)_2D3$ treatment also halted the progression of severe arthritis compared to the controls. Human TNF transgenic mouse is another animal model of spontaneous chronic arthritis. When these mice were crossed with VDR deficient mice, clinical symptoms of arthritis were aggravated. Absent VDR signaling was associated with increased synovial inflammation and macrophage influx into the inflamed joint (Zwerina *et al.*, 2011).

The mechanisms through which vitamin D potentially influences the immune system have been well documented in *in vitro* studies. VDR are present on primary lymphoid organs. Active vitamin D analog inhibits adaptive immunity by suppressing B cell, dendritic cell maturity and T cell proliferation (Maruotti and Cantatore, 2010). Vitamin D has been shown to down regulate selective chemokines and cytokines (Th1 and Th17 cytokines) and to induce T regulatory cells, shifting Th1 responses towards Th2 responses (Bikle, 2010), potentially beneficial for Th1 driven immune diseases. This topic is discussed in greater detail in other chapters of this book. The pathogenesis of RA is complex and involves both T cells and B cells. Antigen presentation to T cells results in their differentiation into Th1 and Th17 cells with secretion of proinflammatory cytokines such as TNF-alpha and IL-17. Studies on healthy controls have shown that $1,25(OH)_2D3$ inhibit the production of TNF-alpha and IL-17 by peripheral blood monocytes (Rausch-Fan *et al.*, 2002). These results were replicated in patients with early RA where presence of activated vitamin D analog resulted in suppression of IL-17 in stimulated PBMC of patients with early RA

(Colin *et al.*, 2010). Thus, vitamin D has the potential to possibly modulate effector pathways that may be critical in pathogenesis of autoimmune arthritis.

Given these findings, it is plausible to hypothesize that vitamin D, through its immunosuppressive effect, may prevent autoimmunity. The simplistic paradigm of initiation of autoimmunity recognizes a 'multi-hit' process, with genetic predisposition resulting in aberrant immune response to an environmental trigger. Is it possible that vitamin D deficiency, via its interaction with the immune system, predisposes to aberrant immune response to an external trigger leading to autoimmune diseases/arthritis? And once autoimmunity is established, can vitamin D deficiency cause exacerbation of inflammatory diseases through its modification of B and T cells and cytokine responses? While epidemiologic, clinical and mechanistic observations point towards a possible role of vitamin D in autoimmunity, conclusive evidence is missing. Moreover, optimal vitamin D levels required to prevent or treat autoimmune disease is unknown.

Clinical studies have shown that low levels of vitamin D are common in RA. While the percentage may vary in different studies depending on the cut-off value used for 25(OH)D levels, several studies indicate greater than 50% prevalence of suboptimal vitamin D levels in this population (Kerr *et al.*, 2011; Rossini *et al.*, 2010). Our data suggest that up to 60% of RA patients have vitamin D deficiency (25(OH)D <30 ng/ml) in the mid-Atlantic region (Haque and Bartlett, 2010). In most studies that compared vitamin D levels in RA patients with controls, no difference was seen between the two groups (Cutolo *et al.*, 2006; Rossini *et al.*, 2010; Turhanoglu *et al.*, 2011), suggesting that RA does not confer an additional risk for vitamin D deficiency.

Patel *et al.* (2007) were the first to report an inverse relationship between vitamin D levels and disease activity and disability in inflammatory arthritis. In a cohort of 206 patients with early inflammatory arthritis (synovitis ≥2 peripheral joints, symptom duration ≥4 weeks), 25(OH)D levels were inversely associated with clinical measures of RA disease activity (tender joint count, CRP and DAS 28). Patients with lower vitamin D levels also had greater disability, as measure by health assessment questionnaire score.

Since then several studies from different parts of the world have evaluated this relationship in patients with RA. In the largest study from Italy, 1,191 patients with RA were examined (Rossini *et al.*, 2010). This sample concerned mostly female patients (85%), with a mean age of 58.9±11.1 years and a disease duration of 11.5±8.7 years. 45% of the study patients were on vitamin D supplements. Prevalence of vitamin D deficiency (25(OH)D <20 ng/ml) was 43% in the entire cohort. One third of patients taking >800 IU vitamin D on enrollment were still deficient. Low 25(OH)D levels were associated with high disease activity (DAS 28) and disability index (health assessment questionnaire), in non-supplemented RA patients. This remained significant even after adjusting for BMI and sun exposure. Similar results were noted in several smaller studies (Cutolo *et al.*, 2006; Haque and Bartlett, 2010; Turhanoglu *et al.*, 2011).

Not all studies, however, have confirmed these results. Kerr *et al.* (2011) evaluated this relationship in 850 men (76% Caucasian) enrolled in the Veterans Affairs Rheumatoid Arthritis Registry.

Similar to earlier studies, 43% of the cohort was vitamin D deficient [25(OH)D <20 ng/ml]. They noted an inverse relation between vitamin D deficiency and tender joint count [OR 1.02, (1.01-1.040, *P*=0.001] and high sensitive CRP [OR 0.99 (0.99-1.00), *P*=0.008] and anti-ccp positivity [OR 1.55, (1.18-2.05), *P*=0.002]. However, no correlation of low vitamin D levels with composite scores of disease activity or disability was observed. Craig *et al.* (2010) also did not observe any relationship between 25(OH)D levels and RA activity in African American patients with early RA.

While these preliminary studies point towards a possible link between vitamin D and RA, it is important to recognize that these are all cross-sectional analyses. This precludes inference about causality. Thus, we are unable to determine whether vitamin D deficiency directly causes increased disease activity in RA or whether the reverse is true. These studies also rely on vitamin D levels measured at one point only. This solitary measure may not be a true reflection of long term vitamin D status in these individuals, as 25(OH)D levels can fluctuate (Dawson-Hughes *et al.*, 1991). Regardless, these data suggest need for larger, prospective interventional studies to replicate these findings and to explore the possible therapeutic role of vitamin D repletion in RA.

Two recent publications have evaluated this question. In a double blind study, patients with active RA (DAS 28 >3.2) on stable disease modifying anti-rheumatic drugs were randomized to 50,000 IU vitamin D weekly or placebo for 12 weeks, on background MTX (Salesi and Farajzadegan, 2011). The primary endpoint was decrease in DAS 28 of 0.6 at week 12. The two groups were similar in demographic and RA related characteristics at baseline. 98 patients completed this study. At week 12, both treatment and placebo groups achieved similar decline in DAS 28, tender joint counts, swollen joint counts and ESR. The only statistical difference observed between the control and active treatment groups was the 25(OH)D levels before and after intervention. It is however, unclear why these two groups showed a robust DAS 28 response rate (placebo group = 64% response rate, vitamin D group = 76%) if no adjustments of disease modifying anti-rheumatic drugs or other medications were allowed that could potentially reduce disease activity, during the study period.

In a larger open label trial, 121 patients with early RA (duration <2 years) were randomized to receive 500 IU $1,25(OH)_3D3$ + 1000 mg calcium carbonate vs. 1000 mg calcium carbonate daily (Gopinath and Danda, 2011). In addition, both groups also received triple therapy (MTX, sulfasalazine, hydroxychloroquine) for 3 months. Baseline demographics and RA characteristics were comparable in the two groups. Primary outcome was minimum time required for onset of pain relief. At study completion, median time to first achieve pain relief was equal in both groups (*P*=0.4). However, patients in the treatment group achieved higher pain relief as measured by visual analog score at the end of 3 months (50% vs. 30%, *P*=0.006). 25(OH)D levels were not measured in all patients and thus the direct correlation of vitamin D levels with pain relief could not be established.

With existing data, the role of vitamin D supplementation in RA, if any is uncertain. Clearly larger, randomized placebo controlled trials of longer duration are needed to evaluate the role of

vitamin D therapy in RA, with assessment of 25(OH)D levels as well as measures of RA disease activity, inflammatory marker and cytokines, at multiple points throughout the study period. This will help us better understand the true relationship of 25(OH)D levels with RA disease activity and its correlation with markers of inflammation and inflammatory cytokines. It may also shed light on the greatest challenge in vitamin D research – the optimal level of 25(OH)D in autoimmune diseases.

Currently there is no expert consensus on the minimum physiologic 25(OH)D levels required for our species. It is difficult to establish a 'norm' as we are evaluating a relatively sun-deprived population. The threshold level for vitamin D deficiency has focused on its skeletal benefits and the need to prevent secondary hyperparathyroidism (Chapuy *et al.*, 1997; Dawson-Hughes *et al.*, 2005). The Institute of Medicine, after evaluating data regarding bone health and vitamin D, proposed a cut-off level of 20 ng/ml. Other experts do not concur with this assessment (Dawson-Hughes *et al.*, 2005). Similarly, the optimal levels of vitamin D required to achieve its possible benefits in autoimmune and other diseases are unknown.

8.3 Vitamin D and osteoarthritis

OA is the most common form of arthritis associated with musculoskeletal pain and disability. It is degenerative arthritis, characterized by loss of articular cartilage with concurrent changes in the subchondral bone (Mansell *et al.*, 1997). Bone mineral density and other characteristics of the subchondral bone may influence the expression and progression of OA (Westacott *et al.*, 1997). Vitamin D influences bone quality by its effect on calcium metabolism, bone density and matrix ossification (Parfitt *et al.*, 1982). Thus, it has been hypothesized that vitamin D can impact development or progression of OA. Several longitudinal studies have explored this possibility, with conflicting results. In the Framingham Heart Study, risk of knee OA progression was three times higher in participants in the middle and lower tertiles for vitamin D intake and 25(OH)D levels compared with the upper tertile (McAlindon *et al.*, 1996). However, no effect on incident knee OA was observed. On the other hand, in the Study of Osteoporotic Fractures, lowest tertile of 25(OH)D was associated with risk of incident hip OA (OR 3.3, 95% CI 1.13-9.86), defined as development of joint space loss. A follow up study evaluating two longitudinal cohorts, reported no association of 25(OH)D levels with progression of knee OA (Felson *et al.*, 2007). Another prospective study evaluated the association of serum 25(OH)D levels, sunlight exposure and knee cartilage loss by MRI and noted that baseline 25(OH)D level were positively associated with cartilage volume and predicted knee cartilage loss over 3 years of follow up (Ding *et al.*, 2009). A recent cross-sectional study noted an association between knee pain and low levels of vitamin D but not with radiographic knee OA (Muraki *et al.*, 2011). These studies present interesting associations but other studies have not replicated these findings (Kalichman and Kobyliansky, 2012; Konstari *et al.*, 2012). Most of these studies used only one point assessment of 25(OH)D levels over several years of follow up, which may not be a true indicator of vitamin D status over years. Several of these studies also used X-rays to evaluate incident or progressive OA, which may be insensitive and slow to change. Thus, at this point, the causal role of vitamin D in the

development and progression of OA is unclear. Further studies are required to assess the potential therapeutic role of vitamin D in OA.

8.4 Conclusion

In conclusion, there is emerging evidence regarding several non-calcemic biological functions of vitamin D, including its immunomodulatory effects. There is epidemiologic, experimental and clinical data that suggest a possible link between vitamin D and arthritis, both inflammatory and degenerative arthritis. While data from studies evaluating the relationship of vitamin D with disease activity in RA and incident and progressive OA are intriguing, the causative role of vitamin D in initiation or exacerbation of arthritis is yet to be established. Most of these studies are cross-sectional and thus the direction of association cannot be determined. It is possible that vitamin D is a surrogate marker of health. Currently, there is no consensus regarding the optimal 25(OH)D levels required to achieve beneficial effects of vitamin D in autoimmune diseases. The role of vitamin D as a preventive or therapeutic agent in chronic arthritis, while a novel prospect, is currently unclear. We await larger, prospective controlled studies to further explore these preliminary findings.

References

Apperly, F.L. and Cary, M.K., 1945. The deterrent effect of light upon the incidence of spontaneous mammary cancer in mice. Cancer Research 5, 697-704.

Bikle, D.D., 2010. Vitamin D: newly discovered actions require reconsideration of physiologic requirements. Trends in endocrinology and metabolism 21, 375-384.

Cantorna, M.T., Hayes, C.E. and DeLuca, H.F., 1998. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. The Journal of Nutrition 128, 68-72.

Cantorna, M.T., Zhu, Y., Froicu, M. and Wittke, A., 2004. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. The American Journal of Clinical Nutrition 80, 1717S-1720S.

Chapuy, M.C., Preziosi, P., Maamer, M., Arnaud, S., Galan, P., Hercberg, S. and Meunier, P.J., 1997. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis International 7, 439-443.

Colin, E.M., Asmawidjaja, P.S., Van Hamburg, J.P., Mus, A.M., Van Driel, M., Hazes, J.M., Van Leeuwen, J.P. and Lubberts, E., 2010. 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. Arthritis and Rheumatism 62, 132-142.

Costenbader, K.H., Feskanich, D., Holmes, M., Karlson, E.W. and Benito-Garcia, E., 2008. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. Annals of the Rheumatic Diseases 67, 530-535.

Craig, S.M., Yu, F., Curtis, J.R., Alarcón, G.S., Conn, D.L., Jonas, B., Callahan, L.F., Smith, E.A., Moreland, L.W., Bridges Jr., S.L. and Mikuls, T.R., 2010. Vitamin D status and its associations with disease activity and severity in African Americans with recent-onset rheumatoid arthritis. The Journal of Rheumatology 37, 275-281.

Cutolo, M., Otsa, K., Laas, K., Yprus, M., Lehtme, R., Secchi, M.E., Sulli, A., Paolino, S. and Seriolo, B., 2006. Circannual vitamin d serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. Clinical and Experimental Rheumatology 24, 702-704.

Dawson-Hughes, B., Dallal, G.E., Krall, E.A., Harris, S., Sokoll, L.J. and Falconer, G., 1991. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Annals of Internal Medicine 115, 505-512.

Ding, C., Cicuttini, F., Parameswaran, V., Burgess, J., Quinn, S. and Jones, G., 2009. Serum levels of vitamin D, sunlight exposure, and knee cartilage loss in older adults: the Tasmanian older adult cohort study. Arthritis and Rheumatism 60, 1381-1389.

Dong, X., Craig, T., Xing, N., Bachman, L.A., Paya, C.V., Weih, F., McKean, D.J., Kumar, R. and Griffin, M.D., 2003. Direct transcriptional regulation of RelB by 1alpha,25-dihydroxyvitamin D3 and its analogs: physiologic and therapeutic implications for dendritic cell function. The Journal of Biological Chemistry 278, 49378-49385.

Felson, D.T., Niu, J., Clancy, M., Aliabadi, P., Sack, B., Guermazi, A., Hunter, D.J., Amin, S., Rogers, G. and Booth, S.L., 2007. Low levels of vitamin D and worsening of knee osteoarthritis: results of two longitudinal studies. Arthritis and Rheumatism 56, 129-136.

Gopinath, K. and Danda, D., 2011. Supplementation of 1,25 dihydroxyvitamin D3 in patients with treatment naive early rheumatoid arthritis: a randomised controlled trial. International Journal of Rheumatic Diseases 14, 332-339.

Grant, W.B., 2002. An estimate of premature cancer mortality in the US due to inadequate doses of solar ultraviolet-B radiation. Cancer 94, 1867-1875.

Haque, U.J. and Bartlett, S.J., 2010. Relationships among vitamin D, disease activity, pain and disability in rheumatoid arthritis. Clinical and Experimental Rheumatology 28, 745-747.

Holick, M.F., 2007. Vitamin D deficiency. The New England journal of medicine 357, 266-281.

Kalichman, L. and Kobyliansky, E., 2012. Association between circulatory levels of vitamin D and radiographic hand osteoarthritis. Rheumatology International 32, 253-257.

Kerr, G.S., Sabahi, I., Richards, J.S., Caplan, L., Cannon, G.W., Reimold, A., Thiele, G.M., Johnson, D. and Mikuls, T.R., 2011. Prevalence of vitamin D insufficiency/deficiency in rheumatoid arthritis and associations with disease severity and activity. The Journal of Rheumatology 38, 53-59.

Konstari, S., Paananen, M., Heliövaara, M., Knekt, P., Marniemi, J., Impivaara, O., Arokoski, J. and Karppinen, J., 2012. Association of 25-hydroxyvitamin D with the incidence of knee and hip osteoarthritis: a 22-year follow-up study. Scandinavian Journal of Rheumatology 41, 124-131.

Mansell, J.P., Tarlton, J.F. and Bailey, A.J., 1997. Biochemical evidence for altered subchondral bone collagen metabolism in osteoarthritis of the hip. British Journal of Rheumatology 36, 16-19.

Maruotti, N. and Cantatore, F.P., 2010. Vitamin D and the immune system. The Journal of Rheumatology 37, 491-495.

McAlindon, T.E., Felson, D.T., Zhang, Y., Hannan, M.T., Aliabadi, P., Weissman, B., Rush, D., Wilson, P.W. and Jacques, P., 1996. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. Annals of Internal Medicine 125, 353-359.

Merlino, L.A., Curtis, J., Mikuls, T.R., Cerhan, J.R., Criswell, L.A., Saag, K.G.; Iowa Women's Health Study, 2004. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis and Rheumatism 50, 72-77.

Muraki, S., Dennison, E., Jameson, K., Boucher, B.J., Akune, T., Yoshimura, N., Judge, A., Arden, N.K., Javaid, K. and Cooper, C., 2011. Association of vitamin D status with knee pain and radiographic knee osteoarthritis. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society 19, 1301-1306.

Nielen, M.M., Van Schaardenburg, D., Lems, W.F., Van de Stadt, R.J., De Koning, M.H., Reesink, H.W., Habibuw, M.R., Van der Horst-Bruinsma, I.E., Twisk, J.W. and Dijkmans, B.A., 2006. Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino *et al.* Arthritis and Rheumatism 54, 3719-3720.

Parfitt, A.M., Gallagher, J.C., Heaney, R.P., Johnston, C.C., Neer, R. and Whedon, G.D., 1982. Vitamin D and bone health in the elderly. The American Journal of Clinical Nutrition 36, 1014-1031.

Patel, S., Farragher, T., Berry, J., Bunn, D., Silman, A. and Symmons, D., 2007. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis and Rheumatism 56, 2143-2149.

Ponsonby, A.L., McMichael, A. and Van der Mei, I., 2002. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. Toxicology 181-182, 71-78.

Racovan, M., Walitt, B., Collins, C.E., Pettinger, M., Parks, C.G., Shikany, J.M., Wactawski-Wende, J., Manson, J.E., Moreland, L., Wright, N., Jackson, R. and Howard, B.V., 2011. Calcium and vitamin D supplementation and incident rheumatoid arthritis: the Women's Health Initiative Calcium plus Vitamin D trial. Rheumatology International 32, 3823-3830.

Rausch-Fan, X., Leutmezer, F., Willheim, M., Spittler, A., Bohle, B., Ebner, C., Jensen-Jarolim, E., Boltz-Nitulescu, G., 2002. Regulation of cytokine production in human peripheral blood mononuclear cells and allergen-specific th cell clones by 1alpha,25-dihydroxyvitamin D3. International Archives of Allergy and Immunology 128, 33-41.

Rossini, M., Maddali Bongi, S., La Montagna, G., Minisola, G., Malavolta, N., Bernini, L., Cacace, E., Sinigaglia, L., Di Munno, O. and Adami, S., 2010. Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. Arthritis Research & Therapy 12, R216.

Salesi, M. and Farajzadegan, Z., 2011. Efficacy of Vitamin D in patients with active rheumatoid arthritis receiving methotrexate therapy. Rheumatology International 32, 2129-2133.

Sokka, T. and Pincus, T., 2005. Quantitative joint assessment in rheumatoid arthritis. Clinical and Experimental Rheumatology 23, S58-62.

Sutton, A.L. and MacDonald, P.N., 2003. Vitamin D: more than a 'bone-a-fide' hormone. Molecular Endocrinology (Baltimore, Md.) 17, 777-791.

Turhanoglu, A.D., Güler, H., Yönden, Z., Aslan, F., Mansuroglu, A. and Ozer, C., 2011. The relationship between vitamin D and disease activity and functional health status in rheumatoid arthritis. Rheumatology International 31, 911-914.

Westacott, C.I., Webb, G.R., Warnock, M.G., Sims, J.V. and Elson, C.J., 1997. Alteration of cartilage metabolism by cells from osteoarthritic bone. Arthritis and Rheumatism 40, 1282-1291.

Zwerina, K., Baum, W., Axmann, R., Heiland, G.R., Distler, J.H., Smolen, J., Hayer, S., Zwerina, J. and Schett, G., 2011. Vitamin D receptor regulates TNF-mediated arthritis. Annals of the Rheumatic Diseases 70, 1122-1129.

Key facts

- The ability of aging skin to produce vitamin D is reduced by more than half in 80 year olds compared to adolescents, due to reduced epidermal production efficiency.
- Exposure of the body in a bathing suit to sunlight for around 6-9 minutes (assuming a fair-skinned person exposed to midday summer sun in temperate regions) is equivalent to an oral dose of around 75 µg (3,000 IU) vitamin D.
- Vitamin D that is not attained from sunlight cannot realistically be replaced by a diet that includes only normal foodstuffs.
- Following an oral dose of vitamin D, 25-hydroxyvitamin D [25(OH)D] reaches peak circulating level in 2-3 weeks, and disappears with a half-life of around 1-3 months.
- The dose-response of 25(OH)D to vitamin D is often estimated as being around 1 nmol/l per µg/day vitamin D (2.5 nmol/l per 100 IU/day or 0.4 ng/ml per µg/day) but is highly variable and depends on a range of factors.

Summary points

- Elderly people often have low vitamin D status due to reduced sunlight exposure and physiological changes in epidermal vitamin D production.
- Research surrounding the use of high-dose vitamin D has expanded since the mid-2000s.
- Moderate- and high-dose interventions include sustained doses ≥25 µg/day (1000 IU/day) or equivalent administered weekly or monthly and higher seasonal, annual or single doses.
- Efficacy of moderate- and high-dose vitamin D depends on factors affecting the pharmacodynamic response of 25(OH)D to vitamin D supplementation, including initial vitamin D status, body composition, form of vitamin D and the dose itself.
- Studies investigating efficacy of moderate- and high-dose vitamin D interventions on musculoskeletal (fractures, falls and their determinants), and other outcomes are inconclusive.
- Sustained daily doses of almost 250 µg/day (10,000 IU/day) and single doses up to 15 mg (600,000 IU) vitamin D have been used in trials involving elderly participants.
- Two studies of daily doses above 160 µg/day (6,500 IU/day) report elevated calcium and episodes of hypercalcaemia.
- Two recent studies suggest that large annual doses of 7.5-12.5 mg (300,000-500,000 IU) may increase risk of fractures and falls.
- Other extra-skeletal benefits of high vitamin D status are proposed but not well supported by data from randomized-controlled trials.

9. High-dose vitamin D supplementation in the elderly

C.J. Bacon
Department of General Practice and Primary Health Care, School of Population Health, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; c.j.bacon@fitkiwi.co.nz

Abstract

The vast majority of studies assessing the effect of vitamin D on a range of outcomes, provide doses of <25 μg/day to older adults. Because of the potential for enhanced convenience or compliance, there has been an interest in higher doses, though availability of high dose products in many parts of the world, particularly Australia and North America, has limited their clinical applicability. Since the middle of the last decade, there has been a rapid expansion of data surrounding efficacy and safety of high-dose interventions, in elderly and in other population groups. Such regimens include weekly or monthly doses, and larger doses administered seasonally, annually, or as loading doses to rapidly boost vitamin D status. The efficacy of high-dose vitamin D supplementation over lower-dose preparations depends on the 25-hydroxyvitamin D response to vitamin D supplementation. An examination of data surrounding the pharmacodynamic effect of oral vitamin D shows that the dose-response is highly variable and depends on factors such as basal vitamin D status, body composition, the type of vitamin D provided and the dose itself. Controlled interventions examining the efficacy of high doses in elderly have mainly investigated musculoskeletal outcomes. Evidence of high-dose vitamin D efficacy is inconclusive for fracture- and falls-risk, their musculoskeletal determinants in this population group, and for other outcomes. Whilst most studies that provide high doses of vitamin D and report follow-up measurements of calcaemic effects find little evidence of increased hypercalcemia, hypercalciuria or renal stones, relative to control groups, there are a lack of adverse effect data from large controlled investigations. A cautious approach to administering loading doses ≥7.5 mg of vitamin D might be warranted since two recent studies have shown modestly increased risk of falls and hip fractures.

Keywords: calciferol, cholecalciferol, ergocalciferol, aged

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_9, © Wageningen Academic Publishers 2013

C.J. Bacon

Abbreviations

25(OH)D	25-hydroxyvitamin D
MED	Minimal erythemal dose
UL	Tolerable upper intake level

Unit conversions

25(OH)D concentration	1 nmol/l = 0.40 μg/ml
Vitamin D dose	1 μg = 40 IU (100,000 IU = 2.5 mg)

9.1 Introduction

Low levels of circulating 25(OH)D are common in frail elderly people (Lips, 2007; McKenna, 1992) for a combination of physiological and behavioral reasons. The ability of aging skin to produce vitamin D is reduced by more than half in 80 year olds compared to adolescents, due to lowered epidermal 7-dehydrocholesterol concentrations (MacLaughlin and Holick, 1985) and increased skin thickness (Need *et al.*, 1993). The effects of aging may also reduce mobility, and physical activity levels are inversely related to vitamin D status in elderly (Bolland *et al.*, 2006; Lucas *et al.*, 2005). Low vitamin D status associated with inactivity is likely to result primarily from reduced outdoor time and increased clothing necessary for warmth as a consequence of impaired mobility (Holick, 1995). Other behavioral factors may also contribute, including deliberate avoidance of direct sun exposure, for example to reduce the risk or exacerbation of skin cancer or for cosmetic protection (Armstrong, 2004; Gilchrest, 2008; Webb, 2006).

Replacement of vitamin D that is not attained from sunlight by consuming normal foodstuffs is not realistic. Exposure of the body in a bathing suit to a modest 0.3 MED of ultra-violet, equivalent to one third the amount required to cause minimal redness of the skin, or 6-9 minutes of sun exposure for a fair skinned person in midday summer sun in temperate regions (Ebeling and Eisman, 2005), is equivalent to an oral dose of around 75 μg vitamin D (Holick, 2001, 2007). By comparison, this dose would be obtained from approximately ten 100 g cans of sardines, 100 eggs, 300 litres unfortified, or 7 litres fortified full-cream milk (Holick, 2007; New Zealand Institute for Crop and Food Research Ltd., 2004). Even for the richest natural source of vitamin D, cod liver oil, 15 teaspoons would be required. Vitamin D fortification of food is also rare in Southern Europe, the United Kingdom, Australia and New Zealand. Consequently, because minimal vitamin D is obtained from any normal diet, it is clear that individuals who do not obtain exposure to outdoor sun require pharmacologically administered vitamin D.

There is substantial uncertainty about what dose to prescribe elderly people who may have low vitamin D status due to lack of sunshine exposure. This uncertainty comes about mainly because of disagreement about what an optimal target level of circulating 25(OH)D should be (Bouillon,

2011; Dawson-Hughes *et al.*, 2005; Lanham-New *et al.*, 2011; Vieth, 2011) and what is the safe upper limit for vitamin D dose (Aloia, 2011; Vieth, 2007). There is no clearly defined threshold level that constitutes a high-dose of vitamin D, and the term *high-dose* has been variously used to describe daily doses of between 50-2,200 µg (Grimnes *et al.*, 2012; Hackman *et al.*, 2010; Hypponen *et al.*, 2011; Kimball and Vieth, 2008); weekly doses of 35-5,000 µg (Maalouf *et al.*, 2008; Wasse *et al.*, 2012); monthly doses of 1,250 µg (Bacon *et al.*, 2009); and single or annual doses of 7,500-15,000 µg (Bacon *et al.*, 2009; Cesur *et al.*, 2003; Sanders *et al.*, 2010; Von Restorff *et al.*, 2009; Wu *et al.*, 2003). Analysis of studies from a number of meta-analyses show that the vast majority of randomized controlled trials of vitamin D2 or D3, alone or in combination with calcium, to prevent osteoporosis, fractures or falls in elderly, have provided daily doses of 7.5-20 µg (Bischoff-Ferrari *et al.*, 2009; Cranney *et al.*, 2008; Tang *et al.*, 2007). There has been recent interest in studies reporting on higher and/or less frequent vitamin D dose regimens given to this population group. Trials administering vitamin D in this way could be broadly classified into five groups:

1. Daily moderate dose: 25-50 µg/day (1000-2,000 IU/day);
2. Daily high dose: >50 µg/day (>2,000 IU/day);
3. Weekly moderate dose: 250-375 µg/day (10,000-15,000 IU/week);
4. Weekly high dose >375 µg/day (>15,000 IU/week);
5. Loading dose, monthly or seasonal dose.

The aim of this chapter is to address the evidence surrounding moderate- and high-dose vitamin D administration in elderly including the pharmacodynamics, efficacy, potential benefits and possible risks.

9.2 Pharmacodynamics of vitamin D

Circulating 25(OH)D has a relatively lengthy half-life, variably estimated between 10 and 27 days following its own oral or intravenous administration. This half-life depends upon its initial levels (Haddad and Rojanasathit, 1976), dietary fiber intake (Batchelor and Compston, 1983), method of determination (Vicchio *et al.*, 1993), and potentially levels of vitamin D binding protein (Christakos *et al.*, 2003). It is important to recognize that the pharmacokinetics of administered 25(OH)D is not equivalent to the time-course of 25(OH)D following an oral dose of vitamin D3. The latter depends on the absorption of vitamin D3, the hepatic conversion of vitamin D3 to 25(OH)D, the degree of initial accumulation or storage of vitamin D3 in body tissues and, in addition, to the catabolic processes responsible for 25(OH)D break-down, which determine its disappearance half-life. Based on the earlier physiological studies of Mawer *et al.* (1972), Vieth (2008) has estimated a half-life of combined vitamin D metabolites of around 2 months, somewhat longer than for 25(OH)D half-life.

Until quite recently, there has been little empirical data to assess the pharmacodynamic effect on 25(OH)D of different vitamin D doses. Whilst a number of studies report baseline and single follow-up measurements of 25(OH)D following vitamin D administration in elderly, (for

example, Aloia *et al.*, 2005; Guillemant *et al.*, 1989; Khaw *et al.*, 1994; von Hurst *et al.*, 2010), fewer have reported serial follow-up measures of 25(OH)D (Table 9.1). Data from these studies can be used for two purposes relevant to evaluating the effects of high-dose vitamin D administration. Firstly, those assessing 25(OH)D concentration following cessation of vitamin D or after the administration of a single high dose can confirm the half-life for disappearance of 25(OH)D in the clinical situation. Secondly, data from studies measuring the rise in 25(OH)D resulting from sustained doses of vitamin D, of sufficient duration to attain a new steady-state, can be used to estimate doses required to attain or maintain target levels of 25(OH)D.

9.2.1 Clinical half-life of 25-hydroxyvitamin D

Vieth's (2008) estimate of 2 months for the half-life of combined vitamin D metabolites was confirmed in a US study of healthy American adults aged 27-84 years following a 2.5 mg acute dose of vitamin D3 (Ilahi *et al.*, 2008) which showed that peak circulating 25(OH)D occurred at 7 days with an ensuing linear decline that displayed a half-life of approximately 50 days. Two New Zealand studies report data from similar investigations administering vitamin D3 to elderly men and women recruited from hospital wards (Bacon *et al.*, 2009; Wu *et al.*, 2003). One of these studies noted a longer 25(OH)D half-life of 90 days following a peak response to 7.5 mg vitamin D3 that occurred between 13 and 21 days (Wu *et al.*, 2003) and the other reported a half-life of 1.3 months after a peak level observed at the first follow-up visit at 1 month, in a group who received a single dose of 12.5 mg vitamin D3 (Bacon *et al.*, 2009). Since the actual peak 25(OH)D in the latter study (Bacon *et al.*, 2009) might have occurred 1-3 weeks earlier than the 1-month measurement, the observed half-life is not inconsistent with that observed in the US study (Ilahi *et al.*, 2008). The longer half-life in the former New Zealand study (Wu *et al.*, 2003) may simply reflect greater levels of sun exposure in the study group during follow-up. Whilst these data suggest a clinically-applicable 25(OH)D half-life of around 2-3 months, it is possible that the half-life for these large acute doses is longer than it would be for smaller doses. Higher doses may result in greater accumulation and storage of circulating vitamin D3 and more sustained subsequent release from storage depots in concentrations below the saturation level of hepatic hydroxylation enzymes, and consequently greater overall conversion to 25(OH)D.

9.2.2 25-hydroxyvitamin D response to sustained doses

Data from studies that measure 25(OH)D concentration before and after sustained vitamin D supplementation have been used to calculate a response per unit dose (dose-response). An approximate figure of 1 nmol/l increase for every 1 μg vitamin D (2.5 nmol/l per 100 IU) has been suggested (Holick, 2008; Vieth, 2007), but the 25(OH)D dose-response varies widely (Barger-Lux *et al.*, 1998; Binkley *et al.*, 2011; Gallagher *et al.*, 2012) ranging from 0.22-3.1 nmol/l per μg for dose comparison studies in Table 9.1. The actual dose-response may be underestimated for most studies in Table 9.1 since vitamin D treatment periods are relatively short (≤6 months) and a plateau in 25(OH)D may not have occurred. Normally, a duration of 4.5 times the half-life, perhaps 9 months according to Vieth's (2008) estimate above would be considered adequate for attaining a new steady state (Buxton, 2006).

Table 9.1. Response of 25-hydroxyvitamin D following vitamin D supplementation in adults. Data are mean ± SD unless indicated otherwise.

Study	Intervention(s) (µg)	Duration (weeks)	Participants: n; age (y)[1]	Baseline 25(OH)D (nmol/l)	Peak 25(OH)D response[2] (nmol/l) or dose-response (nmol/l per µg/day)
Acute (single dose) – Studies reporting serial 25(OH)D measurements					
Davies *et al.* (1985)	2,500 D2/6 mth	12	128 elderly	6 est. (sample n=20)	not reported
	2,500 D2/y				
Weisman *et al.* (1986)	2,500 D	17-22	27 elderly; range: 72-94	56	peak at 2 weeks
Wu *et al.* (2003)	7,500 D3	≤102	49 elderly; 84±5 (range: 69-94)	17±10	62 (est. from figure)
					peak between 13-21 days
					half-life 90 days
Ilahi *et al.* (2008)	2,500 D3	16	30 adults; 20 older 71±6 (range: 61-84)	68±19	37
			10 younger 38±8 (range: 27-47)		peak at 7 days
					half-life[3] approx. 60 days
Premaor *et al.* (2008)	7,500 D3	39	14 elderly; 79±8 whole cohort (n=28):	31±17	50 (est. from figure)
			81±9 (range: 65-102)		peak between 0-2 months
					half-life not reported
Bacon *et al.* (2009)	12,500 D3	39	19 elderly; 83±6 whole cohort (n=63):	58±25	60
			82±7		peak between 0-3 months
					half-life 1.3 months
Steady state (sustained dose) – Studies comparing different doses or dose regimens					
MacLennan & Hamilton (1977)	12.5 D/d	4	elderly hospital patients	7 est. from figure (<15)	0.24
	50 D/d			14 est. from figure (<30)	0.32
Stamp *et al.* (1977)	20-2,500 D	≥17	128 adults mixed ages	not reported	not reported
Barger-Lux *et al.* (1998)	25 D3/d	8	116 young men; 28±4 (range: 20-37)	67±25	0.82[4]
	250 D3/d				0.62[4]
	1,250 D3/d				0.51[4]
Vieth *et al.* (2001)	25 D3/d	9-22	61 adults; 41±9	43±17	1.20
	100 D3/d			38±13	0.60

Table 9.1. Continued.

Study	Intervention(s) (µg)	Duration (weeks)	Participants: n; age (y)[1]	Baseline 25(OH)D (nmol/l)	Peak 25(OH)D dose-response (nmol/l per µg/day)
Steady state (sustained dose) – Studies comparing different doses or dose regimens (continued)					
Heaney *et al.* (2003)	25 D3/d	≈20	67 men; 39±11	70±20	0.57[4]
	125 D3/d				0.66[4]
	250 D3/d				0.58[4]
Viljakainen *et al.* (2006)	5 D3/d	6	49 elderly women; 71±4 (range: 65-85)	46±14	2.18
	10 D3/d			47±10	1.44
	20 D3/d			44±13	1.19
Chel *et al.* (2008)	15 D3/d	17	338 elderly; 84±6	24±9	3.13
	105 D3/wk			27±13	2.66
	450 D3/mth			24±9	1.98[5]
Ish-Shalom *et al.* (2008)	37.5 D3/d	8	48 elderly women; 81±8	38±17	1.20
	262.5 D3/wk			39±25	0.90
	1,125 D3/28 d			40±25	1.30
Sneve *et al.* (2008)[6]	500 D3/wk	52	296 overweight & obese adults; 47	51±18	0.53
	500 D3/2×wk		(range: 20-71) whole cohort (n=445): 48±11	55±17	0.42
Pekkarinen *et al.* (2010)	20 D3/day	52	40 elderly women; 74 (median) (range:	54 (median)	0.83
	2,433 D3/4 mth		69-79)	57.5 (median) (range: 17-84)	0.23[5]
Van Groningen *et al.* (2010)	625 D3/wk	8	208 adults; 55±17 (range: 18-88)	21±8	0.77
	625 D3/wk	6		20±10	0.48
	625 D3/2 wk	8		19±7 (whole cohort <50)	0.66
Binkley *et al.* (2011)	40 D2/d	52	64 elderly; 73±6 est. from table (range:	87±24	0.38
	40 D3/d		65-88)	75±27	0.57
	1,250 D2/mth			81±24	0.22
	1,250 D3/mth			87±23	0.54

Table 9.1. Continued.

Study	Intervention(s) (µg)	Duration (weeks)	Participants: n; age (y)[1]	Baseline 25(OH)D (nmol/l)	Peak 25(OH)D dose-response (nmol/l per µg/day)
Steady state (sustained dose) – Studies comparing different doses or dose regimens (continued)					
Verschueren *et al.* (2011)	20 D3/d	26	113 elderly women; 80 ±5 (all >70)	52 ±32	1.67
	40 D3/d			55 ±36	1.18
Grimnes *et al.* (2012)	10 D3/2× d	52	297 postmenopausal women; 63±7	71±22	0.90
	10 D3/2× d + 500 D3/2× wk		(range: 50-80)	71±23	0.70
Gallagher *et al.* (2012)	10 D3/day	26 & 52	163 postmenopausal women; 67±7	38±11	2.61[7]
	20 D3/day	26 & 52	(range 56-91)	39±10	1.68[7]
	40 D3/day	26 & 52		37±10	1.25[7]
	60 D3/day	26 & 52		38±10	1.02[7]
	80 D3/day	26 & 52		40±8	0.85[7]
	100 D3/day	26 & 52		37±9	0.76[7]
	120 D3/day	26 & 52		39±9 (all <50)	0.64[7]

[1] For all subjects taking all vitamin D doses indicated unless otherwise stated.

[2] For acute (single dose) studies, peak response indicates the change in 25(OH)D (nmol/l) from baseline to peak levels with time to reach peak levels and subsequent disappearance half-life. For steady state (sustained dose) studies, dose-response indicates the dose-adjusted response or rise in 25(OH)D per IU/day vitamin D.

[3] Half-life from these data reported in later analysis by common authors Heaney *et al.* (2008).

[4] Corrected for results of analysis of actual vitamin D levels in tablets.

[5] Different preparation of vitamin D compared to other doses in the same study.

[6] Same cohort as Jorde *et al.* (2010b).

[7] Dose response calculated from quadratic function curve fitted to all data.

Abbreviations as follows: 25(OH)D = 25-hydroxyvitamin D; im = intramuscular; est. = estimated; /2× wk = twice weekly; /2 wk = fortnightly; /4 mth = 4 monthly; /6 mth = 6 monthly.

9.2.3 Determinants of 25-hydroxyvitamin D response

The 25(OH)D response to vitamin D supplementation may depend on initial 25(OH)D concentration, body mass or composition, the dose quantity or frequency, and whether vitamin D2 or D3 is used. An inspection of steady-state studies in Table 9.1 suggests that the dose-response varies according to baseline 25(OH)D, with greater increments in 25(OH)D for the same vitamin D dose when initial levels are lower. For example, six studies administer daily doses of 25-40 μg D3 (Barger-Lux *et al.*, 1998; Binkley *et al.*, 2011; Gallagher *et al.*, 2012; Heaney *et al.*, 2003; Ish-Shalom *et al.*, 2008; Vieth *et al.*, 2001). Those with starting 25(OH)D levels below 50 nmol/l show dose-responses of 1.2-1.25 nmol/l per μg/day (Gallagher *et al.*, 2012; Ish-Shalom *et al.*, 2008; Vieth *et al.*, 2001) whereas lower dose-responses (0.6-0.8 nmol/l per μg/day) were obtained in those starting from higher baseline levels (Barger-Lux *et al.*, 1998; Binkley *et al.*, 2011; Heaney *et al.*, 2003). The relation between baseline 25(OH)D and dose-response was also statistically confirmed in three studies in Table 9.1 (Barger-Lux *et al.*, 1998; Gallagher *et al.*, 2012; Viljakainen *et al.*, 2006). Because initial levels of 25(OH)D affect dose-response, the efficacy of vitamin D supplementation is likely to depend upon season of delivery. Seasonal effects have been reported in studies concerning regular vitamin D intake and doses. In one study, self-reported vitamin D supplementation was shown to raise 25(OH)D levels relative to no supplementation in winter but not summer (Rapuri *et al.*, 2004) and, in another randomized controlled trial, 10 μg/day vitamin D reduced bone loss in postmenopausal women (Dawson-Hughes *et al.*, 1991).

Similarly, body mass or adiposity may affect the 25(OH)D response to supplementation, since increases in serum vitamin D3 following a standardized dose of ultra-violet radiation in obese people have been shown to be half that of matched lean individuals (Wortsman *et al.*, 2000) and body mass index is inversely associated with 25(OH)D levels and their change in response to supplementation (Jorde *et al.*, 2010a). Barger-Lux *et al.* (1998) reported independent effects of body mass index, in addition to initial 25(OH)D levels, on 25(OH)D response to vitamin D3 doses of 25-1,250 μg/day. In contrast, Vieth *et al.* (2001) reported no effect of either initial 25(OH)D concentration or body weight on 25(OH)D change following 5 months of 25-100 μg vitamin D3/day. Similarly, although Gallagher *et al.* (2012) noted lower 25(OH)D levels resulting from supplementation in those with body mass index ≥25 kg/m^2 compared to those below, the difference was consistent across the doses administered, indicating that the dose did not affect the 25(OH)D response. Because 25(OH)D levels are inversely cross-sectionally related to indices of adiposity (Arunabh *et al.*, 2003; Hyppönen and Power, 2007; Liel *et al.*, 1988; Wortsman *et al.*, 2000), it is difficult to determine the relative contribution of lower vitamin D status versus lesser adiposity on elevated 25(OH)D response to vitamin D dose, since low vitamin D status is associated with greater adiposity and they are likely to counteract each other.

Vitamin D dose may also influence 25(OH)D response per unit dose. Heaney *et al.* analyzed data from five studies reporting 25(OH)D response to 18-20 weeks of vitamin D3 treatment (Heaney *et al.*, 2008). Their data suggest that below circulating vitamin D3 levels of 15 nmol/l, estimated as being equivalent to a vitamin D3 input of 50 μg/day, hepatic conversion to 25(OH)D3 depends only on vitamin D3 concentration, and synthesis is close to complete since levels of vitamin D3

remain very low despite increasing 25(OH)D3 levels (Heaney *et al.*, 2008). Above vitamin D3 concentrations of 15 nmol/l, the increase in corresponding steady-state 25(OH)D3 was linear, which the authors attributed to saturation of and rate dependence on hepatic enzymes, combined with a greater degree of production than metabolic consumption. If these data depict the true steady-state relation between circulating vitamin D3 and 25(OH)D3, and equivalent absorption efficiency is assumed, one might expect to observe a lesser 25(OH)D dose-response only when daily vitamin D doses above approximately 50 μg are compared with those below. In contrast to this theoretical expectation, inspection of studies in Table 9.1 suggests that vitamin D dose over a wide range of doses may in fact influence dose-response of 25(OH)D. Of the seven studies in Table 9.1 from which a comparison of the response of different steady-state doses of vitamin D can be made (Barger-Lux *et al.*, 1998; Gallagher *et al.*, 2012; Heaney *et al.*, 2003; Sneve *et al.*, 2008; Van Groningen *et al.*, 2010; Vieth *et al.*, 2001; Viljakainen *et al.*, 2006), all but one (Heaney *et al.*, 2003) demonstrate a trend for a lesser dose-response at higher compared to lower doses. The differences in 25(OH)D dose-response for doses within the range of 5-1,250 μg/day might suggest a continuous reduction in efficiency of absorption of vitamin D or completeness of hepatic synthesis of 25(OH)D3 from vitamin D with increasing doses. Nonetheless, the observed differences in dose-response are likely to be relatively small in relation to variability between individuals and in the only of these studies to provide statistical reporting of such a comparison, differences between doses did not attain statistical significance (Viljakainen *et al.*, 2006).

The response of 25(OH)D may also be affected by the frequency of delivery of vitamin D. Theoretically, vitamin D3 doses administered at approximately the frequency of the half-life for vitamin D metabolites would be expected to have similar effects to same cumulative dose administered daily (Buxton, 2006). If the half-life of 25(OH)D following vitamin D administration is in the order of 2 months as suggested by Vieth (2008), then very little difference in 25(OH)D response would therefore be expected when equivalent doses of vitamin D are administered daily, weekly or monthly. Two recent studies addressing whether this is so provide conflicting data. Confirming the theoretical expectation, Ish-Shalom *et al.* (2008) administered daily, weekly and monthly (28-day) vitamin D3 at doses equivalent to 37.5 μg/day to elderly women and demonstrated no appreciable difference in the rise in 25(OH)D levels over 56 days. In contrast, Chel *et al.* (2008), who also compared the effects on 25(OH)D of 15 μg/day vitamin D3 and equivalent weekly or monthly doses, found progressively smaller rises in 25(OH)D, after 4 months, from daily to weekly to monthly dosing. Though statistically significant, 25(OH)D response for daily dosing was only 6.5 nmol/l greater than for weekly dosing, a difference which is probably clinically unimportant. Reasons for the different results between the two studies are unclear. In a follow-up comment on the Chel *et al.* (2008) study, Vieth (an author in the Ish-Shalom *et al.*, 2008 study) suggested that differences could have resulted from the use of a powdered preparation for the monthly dose only, which may have been incompletely consumed, or from lower 'trough' 25(OH)D concentrations for higher, less frequent doses (Vieth, 2008).

The form of administered vitamin D, that is whether D2 (ergocalciferol) or D3 (cholecalciferol) is taken, may be another determinant of 25(OH)D dose-response. Based on the treatment of rickets from the 1930s, vitamins D2 and D3 have traditionally been considered to be equally

effective in humans (Armas *et al.*, 2004) and nutritional guidelines do not differentiate between the two (Vieth, 2004). However, more recent work has suggested a quantitative difference in 25(OH)D rise following supplementation. A number of studies have shown that vitamin D3 compared to D2 results in 0.5-2.5 fold greater increases in 25(OH)D levels (Binkley *et al.*, 2011; Tjellesen *et al.*, 1986; Trang *et al.*, 1998) or up to 9.5 times greater when the area under the curve is considered (Armas *et al.*, 2004). Confirming these findings, a South American study reported 3-month 25(OH)D responses to 125-250 μg/day vitamin D2 supplementation relative to a control group of 0.2 nmol/l per μg/day, or around a third of that expected for comparable trials of vitamin D3 (Mastaglia *et al.*, 2006). On the other hand, recent data reported by Holick and coworkers introduce some uncertainty about the relative advantage of vitamin D2 or D3, as these authors failed to find any difference in 25(OH)D response between daily 250 μg doses of either form (Holick *et al.*, 2008). More uncertainty arises from assay-related difficulties, when comparing 25(OH)D response to vitamin D2 and D3 therapies, since some assays unevenly measure 25(OH)D2 and 25(OH)D3 (Hollis, 2000; 2004).

In summary, surprisingly few data inform clinicians about the likely time-course of 25(OH)D response to vitamin D supplementation. Whilst pragmatic figures have been suggested for calculating the likely 25(OH)D response to specific vitamin D doses, these are likely to be highly variable and depend on a range of factors, including vitamin D status, body composition, the form of vitamin D and dose administered. It is possible that high doses of vitamin D (above 50 μg/day or equivalent) result in a different equilibrium relationship between circulating vitamin D and its hydroxylated metabolites. It is important to consider determinants of the 25(OH)D response to vitamin D supplementation since any factor that affects dose-response will also affect its efficacy for health outcomes.

9.3 Efficacy of high-dose vitamin D in elderly

Although the vast majority of studies determining the efficacy of vitamin D supplementation have provided low (<25 μg) daily doses (Avenell *et al.*, 2005; Bischoff-Ferrari *et al.*, 2004, 2005, 2009; Boonen *et al.*, 2006; Cranney *et al.*, 2008; Latham *et al.*, 2003b; Papadimitropoulos *et al.*, 2002; Tang *et al.*, 2007), some contend that this dose is not high enough to meet the needs of elderly people who have limited outdoor sun exposure (Bischoff-Ferrari, 2009; Holick, 2011; Vieth *et al.*, 2007). Indeed, assuming a dose-response of 1 nmol/l per μg/day (a typical value from Table 9.1), a dose of 50 μg/day would be required for an individual with no outdoor sun exposure at all to attain a steady state 25(OH)D level of 50 nmol/l. Furthermore, compliance to daily vitamin D medication may be low in elderly people, particularly if it is deemed to be prophylactic and nutritional in nature, and less essential for health than other medications. Less frequent dose regimens might be more conveniently administered and therefore improve compliance and cost-effectiveness, particularly in hospital or care situations. For these reasons, the data surrounding the efficacy of daily moderate and high doses, and of monthly, seasonal or single loading doses has expanded since the middle of the last decade (Vieth, 2011). One of the challenges for clinicians wishing to adopt these dosing strategies is that Vitamin D3 preparations delivering more than 25

μg per dose have not been readily available in Australia, Europe or North America. Consequently, evaluation of their efficacy when compared to daily low-dose vitamin D has been difficult because whilst most low-dose large studies delivered vitamin D3, until recently the few sizeable high-dose studies that existed were mainly of vitamin D2 (Dhesi *et al.*, 2004; Flicker *et al.*, 2005; Harwood *et al.*, 2004; Heikinheimo *et al.*, 1992; Law *et al.*, 2006; Nordin *et al.*, 1985; Smith *et al.*, 2007).

9.3.1 Musculoskeletal efficacy

Given the preponderance of studies of vitamin D supplementation, relatively few have administered moderate- or high-dose vitamin D to elderly people. A modest number of studies investigating the effects of vitamin D on various musculoskeletal outcomes in elderly people have administered daily or weekly doses equivalent to 25 up to 100 μg/day (Table 9.2). In addition, several studies have administered larger doses, either as a single loading dose, seasonally or annually (Table 9.3). Controlled investigations of the ability of moderate and high doses of vitamin D to reduce fractures in elderly are inconclusive, variously reporting reductions in fractures (Sato *et al.*, 2005; Trivedi *et al.*, 2003), or a reduction in females only (Heikinheimo *et al.*, 1992); no effect on fractures (Law *et al.*, 2006); or, in two recent sizeable studies, an increase in hip (Smith *et al.*, 2007) or all fractures (Sanders *et al.*, 2010). All except one of these studies, which provided 25 μg vitamin D3/day (Sato *et al.*, 2005), administered large infrequent doses ranging from 3.75-12.5 mg/year without additional calcium compared to the control group.

Data determining falls risk-reduction studies recruiting similar populations are also equivocal. Studies that administer moderate daily or weekly vitamin D doses have reported reductions in falls (Flicker *et al.*, 2005; Sato *et al.*, 2005) or in seasonal (winter and spring only) falls (Prince *et al.*, 2008). However, of those giving elderly people large seasonal or annual doses only one study has noted a reduction in falls (Harwood *et al.*, 2004), with others reporting no effect (Latham *et al.*, 2003a; Law *et al.*, 2006; Smith *et al.*, 2007), or increased falls risk (Sanders *et al.*, 2010).

Findings from randomized controlled trials reporting the effects of moderate or high-dose vitamin D on changes in bone mineral density are also inconsistent. Two smaller studies report reduced hip bone mineral density loss following either daily 25 μg vitamin D2 and 1.2 g calcium for 5 years, compared to placebo (Zhu *et al.*, 2008), or 1 year following either a single dose of 7.5 mg vitamin D2 or 20 μg vitamin D3 daily combined with 1 g calcium, with larger effects noted for the latter preparation (Harwood *et al.*, 2004). In contrast, another study investigating the effects on older African American women of 20 μg vitamin D3, which was increased to 50 μg for the third year of the trial, found no effect on either changes in bone mineral density at any site or in bone turnover markers (Aloia *et al.*, 2005). The lack of effect of 15 μg/day vitamin D3 on bone turnover markers was confirmed by Chel *et al.* (2008). Three studies have also failed to show an effect of moderate- (Haworth *et al.*, 2004; Verschueren *et al.*, 2011) to high-dose (Grimnes *et al.*, 2012) vitamin D compared to low doses (20-22.5 μg/day) on bone loss.

A number of studies have assessed the effect of vitamin D on muscle function or functional performance, again with mixed results. Improvements, relative to placebo, in muscle strength and

Table 9.2. Controlled trials with musculoskeletal outcomes administering vitamin D at daily doses ≥1000 IU to elderly people. Data are mean ± SD unless indicated otherwise.

Study	Intervention(s) (μg)[1]	Duration (months)	Participants: n; age (y)[2]	Baseline 25(OH)D (nmol/l)[2]	Main treatment outcome(s)
Johnson *et al.* (1980)	50 D/d	6	120 elderly; (≥65)	not reported	prevented fall in serum phosphate; no effect on muscle function
Corless *et al.* (1985)	225 D2/d	2-9	65 elderly; 82±37	17±12	no effect on activities of daily living or mental function
Nordin *et al.* (1985)	375 D2/wk	24	109 elderly women; 70±2 (range: 65-74)	22±15 [3]	reduced rate of cortical bone loss
Honkanen *et al.* (1990)	45 D3/d + 1 g calcium	2.5 (11 wk)	139 elderly women; 77±8 (≥65) 52 home living: 70±3, 63 hospital: 83±7	home living: 40±16 hospital: 24±12	no effect on grip strength in either group
Kenny *et al.* (2003)	25 D3/d + 0.5 g calcium versus calcium	6	65 elderly men; 76±4 (range: 65-87)	62±18	no effect on muscle strength, power or physical performance
Aloia *et al.* (2005)	20 D3 then 50 D3 for 3rd year + calcium to 1.2–1.5 g total intake versus calcium	36	208 older African American women; 61±6 (range 50-75)	46±19	no effect on bone loss or bone turnover markers
Flicker *et al.* (2005)	250 D2/wk then 25 D2/d	24	625 elderly; 83±8	42 est. from frequency distribution (range: 25-90)	reduced falls rate
Sato *et al.* (2005)	25 D2/d	24	96 elderly women; 74±4	24±3 (all <25)	reduced falls and fractures; increased muscle hypertrophy & strength
Chel *et al.* (2008)	15 D3/d 105 D3/wk 450 D3/mth	4	338 elderly; 84±6	25±11	no effect on bone turnover markers

Table 9.2. Continued.

Study	Intervention(s) (µg)[1]	Duration (months)	Participants: n; age (y)[2]	Baseline 25(OH)D (nmol/l)[2]	Main treatment outcome(s)
Prince *et al.* (2008) & Zhu *et al.* (2010)	25 D2/d + 1 g calcium vs. calcium	12	302 elderly women; 77±5 (range: 70-90)	45±13 (all <60)	reduced first fall risk in winter & spring; improved timed up-and-go & hip muscle strength for those in lowest outcome tertiles
Zhu *et al.* (2008)	25 D2/d + 1.2 g calcium vs. 2 control groups	60	120 elderly women; 75±3 (range: 70-80)	68±29	reduced hip BMD loss especially in those ≤68 nmol/l
Verschueren *et al.* (2011)	40 D3/d + 1 g calcium vs. 800 D3/d + calcium	6	113 elderly women; 80 ±5 (all >70)	53±34	no difference between doses in hip BMD or in muscle strength or mass
Grimnes *et al.* (2012)	1,140 D3/wk + 0.5 g calcium vs. 20 D3/d + calcium	12	297 postmenopausal women; 63±7 (range: 50-80)	71±23	no difference between doses in BMD change; bone formation marker (P1NP) reduced more in low compared to high dose.

[1] Placebo-controlled unless indicated otherwise.

[2] For all enrolled subjects taking all vitamin D doses and control group(s) unless otherwise stated.

[3] Reported values corrected for assay methodological error used for this calculation.

Abbreviations as follows: 25(OH)D = 25-hydroxyvitamin D; vs. = versus; /d = daily; /wk = weekly; /mth = monthly.

Table 9.3. Controlled trials with musculoskeletal outcomes administering vitamin D in monthly, seasonal or single high doses. Data are mean ± SD unless indicated otherwise.

Study	Intervention(s) (mg)[1]	Duration or follow-up (months)	Participants: n; age (y)[2]	Baseline 25(OH)D (nmol/l)[2]	Main treatment outcome(s)
Heikinheimo *et al.* (1992)	3.75 D2/y im	24-60	799 elderly; 84 (all >75)	20 (subgroup est. from figure in previous report (Heikinheimo *et al.*, 1991))	fewer fractures in females only; fewer upper limb and rib fractures
Latham *et al.* (2003a)	7.5 D3 single	6	243 elderly; 80	42 median est.	no effect on falls of physical health or performance
Trivedi *et al.* (2003)	2.5 D3/4 mth	60	2686 elderly; 75±5 (range: 65-85)	not reported	reduced risk of fractures (especially osteoporotic sites)
Dhesi *et al.* (2004)	15 D2 single	6	139 elderly past fallers; 26 77±6 (all <65)	(all <30)	improved functional performance, reaction time and balance but not strength
Harwood *et al.* (2004)	7.5 D2 single	52	111 elderly women; 81 whole cohort (n=150): 81 (range: 67-92)	29 (range: 10-85)	increased hip BMD and reduced falls
Law *et al.* (2006)	2.5 D2/3 mth	10 median	3717 elderly; 85	59; 47 median (in sample, n=18)	no effect on fall or fracture risk
Smith *et al.* (2007)	7.5 D2/y im	36	9440 elderly; 79 median (all >75)	not reported	increased hip fracture risk; no effect on other fractures or falls
Sanders *et al.* (2010)	12.5 D3/y	36-60	2256 elderly women; 76 median (all >70)	49 median est. (53 median in treatment group)	Increased risk of falls and fractures especially in the first 3 months after dose

[1] Placebo-controlled unless indicated otherwise.

[2] For all enrolled subjects taking all vitamin D doses and control group(s) unless otherwise stated.

Abbreviations as follows: 25(OH)D = 25-hydroxyvitamin D; im = intramuscular; est. = estimated; BMD = bone mineral density; /mth = monthly; /y = yearly.

function with 25 µg/day vitamin D2 and 1.2 g calcium were noted for a subgroup of participants with baseline levels in the lowest tertile (Zhu *et al.*, 2010) and in functional performance tests but not strength, following a single 15 mg vitamin D2 dose, in another study (Dhesi *et al.*, 2004). However, for the majority of similar investigations, no effect on muscle strength or functional performance has been noted for a range of daily moderate-, high- or loading-dose vitamin D preparations, either compared to placebo (Corless *et al.*, 1985; Honkanen *et al.*, 1990; Johnson *et al.*, 1980; Kenny *et al.*, 2003; Latham *et al.*, 2003a; Witham *et al.*, 2010a), or compared to lower doses of vitamin D (Grimnes *et al.*, 2012; Verschueren *et al.*, 2011).

9.3.2 Other effects

A few studies have also investigated the effect of moderate-, high- or loading-dose vitamin D supplementation on a range of non-musculoskeletal indices in elderly, sometimes as secondary or tertiary outcome variables, notably cardiovascular-metabolic variables such as blood pressure, cholesterol, endothelial function, B-type natriuretic peptide, and glucose metabolism (Scragg *et al.*, 1995; Witham *et al.*, 2010a,b); mortality from chronic diseases (Trivedi *et al.*, 2003); mental function and depression (Corless *et al.*, 1985; Jorde *et al.*, 2008); and inflammatory cytokines (Jorde *et al.*, 2010c; Schleithoff *et al.*, 2006). Data from these studies cumulatively note effects in favor of vitamin D only for colon cancer mortality (Trivedi *et al.*, 2003), blood pressure and B-type natriuretic peptide (Witham *et al.*, 2010a,b), cytokines (Schleithoff *et al.*, 2006) and depression (Jorde *et al.*, 2008).

9.4 Safety of high-dose vitamin D in elderly

9.4.1 Vitamin D toxicity

High doses of vitamin D are toxic and may result in hypercalcaemia, renal stones and the calcification of soft tissue and blood vessels (Cranney *et al.*, 2007; Vieth, 2007). Because high doses may accumulate in fat, or possibly muscle, tissue (Heaney *et al.*, 2008; Jones, 2008), the effects of toxicity may be long-lasting. Hypercalcaemia comes about via increased intestinal calcium absorption and induction of bone resorption (Suda *et al.*, 2003). Although it was earlier assumed that rises in $1,25(OH)_2D$ were responsible for the toxicity-associated pathology, the expected substantial elevations in this metabolite have not been consistently demonstrated in cases of toxicity (Jones, 2008). It is now believed that pharmacological concentrations 25(OH)D and/or other vitamin D metabolites exceed the vitamin D binding protein capacity, causing free 25(OH)D or displaced $1,25(OH)_2D$ to enter cells and stimulate gene transcription (Jones, 2008). The lowest levels of 25(OH)D associated with toxicity are 221 nmol/l, although they are normally higher than 500-700 nmol/l (Jones, 2008; Vieth, 1999). By comparison, the highest recorded levels associated with natural sun exposure are 225-274 nmol/l following deliberate ultra-violet exposure (Krause *et al.*, 1998; Vieth, 1999). Vieth (1999) has suggested that homeostatic control mechanisms, potentially hepatic, prevent the large increases in 25(OH)D necessary for

hypercalcaemic toxicity with long term (>1 year) vitamin D intakes less than 250 μg/day, or shorter-term doses of 500-1,250 μg/day.

The maximum daily intake of a nutrient deemed unlikely to pose a risk of adverse health events to almost all individuals is known as the UL (Grosvenor and Smolin, 2002). In 2011, the UL was raised from the 1997 recommendation of 50 μg/day to 100 μg/day (Aloia, 2011; Ross *et al.*, 2011). Some prominent researchers have argued for an upward revision of UL to 250 μg/day (Giovannucci, 2011; Hathcock *et al.*, 2007; Heaney, 2005; Holick, 2011; Vieth, 2007). The basis of these arguments are, firstly, that the UL is not supported by evidence of toxicity with serum 25(OH)D levels less than 250 nmol/l (equivalent to continuing oral daily intakes of 250 μg/day). Secondly, it is argued that the UL is too restrictive to allow research into the efficacy of high doses of vitamin D, which is desirable in the light of evidence showing that optimal levels are higher than previously accepted (Bischoff-Ferrari *et al.*, 2006; Dawson-Hughes *et al.*, 2005; Gorham *et al.*, 2007) considered in conjunction with data showing that prevailing vitamin D status in relation to these levels is too low (Calvo *et al.*, 2005; Rockell *et al.*, 2006; Ruston *et al.*, 2004).

9.4.2 Calcaemic effects of high vitamin D doses

Most evaluations vitamin D safety report incidence of hypercalcaemia, hypercalciuria and kidney stones, though most of the data comes from high dose studies of young healthy adults, children or recently in adults diagnosed with multiple sclerosis (Aloia *et al.*, 2008; Burton *et al.*, 2010; Ilahi *et al.*, 2008; Kimball *et al.*, 2007; Maalouf *et al.*, 2008). In a large commissioned systematic review, Cranney *et al.* (2007, 2008) identified 22 trials which focused on incidence of these adverse events in postmenopausal women and elderly men, following supplementation with doses of vitamin D3 ranging from 10 to 100 μg/day and vitamin D2 ranging from 12.5 to 250 μg/day. From meta-analysis, they noted no statistical difference in the incidence of hypercalcaemia or hypercalciuria between treatment and control groups. Reporting of hypercalcaemia was most common and was often explained by other uncovered medical conditions (Cranney *et al.*, 2007). In one study neither serum calcium nor urinary calcium-creatinine excretion ratios changed from baseline in healthy adults provided 100 μg vitamin D3 daily for 6 months (Vieth *et al.*, 2001). Similarly, a later study noted no cases of hypercalcaemia following 6 months of increasing vitamin D3 doses, starting at 50 to 100 μg/day and adjusted at 2-month intervals according to assayed 25(OH)D, to reach a median daily dose of 95 μg (Aloia *et al.*, 2008). Although these authors identified four cases of elevated calcium/creatinine ratio, there was no difference in the number of cases between treatment and placebo groups, and in three cases the elevation was temporary and within the normal range 1 week later (Aloia *et al.*, 2008).

A few studies document adverse events in large vitamin D supplementation studies in postmenopausal women. Some prominence is given to the Women's Health Initiative trial finding of an increased 7-year occurrence of renal stones amongst the treatment group (2.7%) compared to controls (2.3%) from a total of 36,282 postmenopausal women participants (Jackson *et al.*, 2006). However, in addition to 10 μg/day vitamin D3, this study also provided 1 g/day supplemental calcium to participants, so it is not possible to distinguish between the effects

of the two nutrients. In more recent trials of high dose (25 μg/day) vitamin D, hypercalcaemic episodes were transient (Prince *et al.*, 2008) and not different between treatment and control groups (Prince *et al.*, 2008; Zhu *et al.*, 2008).

Despite the lack of documentation of any evidence of adverse calcaemic effects from moderate or high vitamin D doses, frail elderly people may be more at risk from small fluctuations in calcium homeostasis, as a consequence of hepatic, renal or skeletal stress. In particular, there is some concern about the increased potential for adverse effects from large infrequent, or single doses. An Australian study which treated 50 men and women who had low vitamin D status (25(OH)D <50 nmol/l) with intramuscular 15 mg vitamin D3, found that at 12 months, 20% had hypercalciuria (urine calcium/creatinine excretion index of 0.6 or greater), and 4% had mild hypercalcaemia (mean level 2.67 mmol/l compared to upper reference range of 2.65 mmol/l) (Diamond *et al.*, 2005). Nonetheless, it is not clear whether these observations resulted from the vitamin D intervention. Because it would have been unethical to fail to provide vitamin D to individuals with pre-established insufficiency, there was no control group in this study. Furthermore, three individuals who developed hypercalciuria (6% total) also had elevated values at baseline, and primary hyperparathyroidism was uncovered in an additional person. Finally, since the time to peak 25(OH)D after oral administration of a similarly high dose (Wu *et al.*, 2003) is in the region of 2-3 weeks, it is unclear why calcium levels at 12 months but not at 4 months were higher than baseline (Diamond *et al.*, 2005).

9.4.3 Other risks for elderly people

The additional possibility of increased fracture rates following high-dose vitamin D is suggested by the results of a large (n=9,440) intervention in which annual 12.5 mg intramuscular vitamin D2 was provided for 3 years (Smith *et al.*, 2007). In this study, a 49% increased rate of hip or femur fractures was found for those randomized to vitamin D, an effect that extended to any non-vertebral fracture in women (Smith *et al.*, 2007). More recently, an Australian group confirmed these concerns reporting a 4-year 15% increase in falls risk and a 26% increased risk of fractures following annual 15 mg vitamin D3 (Sanders *et al.*, 2010). These effects remained when risk was adjusted for calcium intake, although the adjusted risk of fractures marginally failed to attain statistical significance. Risks of falls and fractures compared to placebo were higher in the first 3 months after each treatment (31% and 53% respectively) than in the subsequent 9 months. The physiological mechanism behind an effect of large doses of vitamin D on falls and fractures is unclear. It may be that the effects on skeletal bone resorption dominate in response to very large single vitamin D doses. In an editorial that accompanied the Australian study report Dawson-Hughes and Harris (2010) speculate that the high dose may have up-regulated $1,25(OH)_2D$ catabolic enzyme as has been observed for 1,875 μg/week vitamin D3 doses in rats (Beckman *et al.*, 1995). It should be noted that this dose in rats is probably equivalent to a per-kg dose in humans approximately 10-fold greater than that delivered to participants in the Australian study (Sanders *et al.*, 2010). Dawson-Hughes and Harris alternatively suggest that the high dose vitamin D may have transiently improved mobility, perhaps via its effect on muscle function, physical performance, pain or mood, which may in turn have increased opportunities for falls to

occur (Dawson-Hughes and Harris, 2010). Whatever the mechanism by which increased falls and fractures in response to very high single doses of vitamin D might occur, it would seem prudent to avoid such high doses, at least until more data documenting adverse events in controlled trials of high-dose vitamin D are available.

9.5 Summary

There has been recent interest in data surrounding the efficacy and safety of vitamin D administered in sustained doses of 25 μg/day and above, equivalent weekly or monthly doses, or as higher seasonal, annual or loading doses. Seasonal or annual doses may be particularly appealing for treatment for frail or institutionalized elderly people because they may be more convenient or improve compliance. The efficacy of these doses is largely dependent on the physiological response of 25(OH)D to vitamin D. The time-course of 25(OH)D changes in response to vitamin D is highly variable and depends on a number of factors that probably include initial vitamin D status, body composition, and the form of vitamin D provided, and potentially include the quantity and frequency of the dose. Whilst many contend that doses up to 10,000 IU/day and equivalent weekly or monthly doses, which probably have a comparable physiological effect, are safe in that the hypercalcaemia of vitamin D toxicity is avoided, limited adverse event data are available from large placebo-controlled studies of elderly people. Caution might be warranted for large single doses of 12.5 mg and above since two recent studies have shown small but statistically significant increases in fall and fracture risk.

References

Aloia, J.F., 2011. Clinical review: the 2011 report on dietary reference intake for vitamin D: where do we go from here? Journal of Clinical Endocrinology & Metabolism 96, 2987-2996.

Aloia, J.F., Patel, M., Dimaano, R., Li-Ng, M., Talwar, S.A., Mikhail, M., Pollack, S. and Yeh, J.K., 2008. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. American Journal of Clinical Nutrition 87, 1952-1958.

Aloia, J.F., Talwar, S.A., Pollack, S. and Yeh, J., 2005. A randomized controlled trial of vitamin D3 supplementation in African American women. Archives of Internal Medicine 165, 1618-1623.

Armas, L.A., Hollis, B.W. and Heaney, R.P., 2004. Vitamin D2 is much less effective than vitamin D3 in humans. Journal of Clinical Endocrinology & Metabolism 89, 5387-5391.

Armstrong, B.K., 2004. How sun exposure causes skin cancer: an epidemiological perspective. In: D. Hill, J.M. Elwood and D.R. English (eds.), Prevention of skin cancer. Kluwer Academic Publishers, Amsterdam, pp. 89-116.

Arunabh, S., Pollack, S., Yeh, J. and Aloia, J.F., 2003. Body fat content and 25-hydroxyvitamin D levels in healthy women. Journal of Clinical Endocrinology & Metabolism 88, 157-161.

Avenell, A., Gillespie, W.J., Gillespie, L.D. and O'Connell, D.L., 2005. Vitamin D and vitamin D analogues for preventing fractures associated with involutional and post-menopausal osteoporosis. Cochrane Database of Systematic Reviews, CD000227.

Bacon, C.J., Gamble, G.D., Horne, A.M., Scott, M.A. and Reid, I.R., 2009. High-dose oral vitamin D3 supplementation in the elderly. Osteoporosis International 20, 1407-1415.

Barger-Lux, M.J., Heaney, R.P., Dowell, S., Chen, T.C. and Holick, M.F., 1998. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporosis International 8, 222-230.

Batchelor, A.J. and Compston, J.E., 1983. Reduced plasma half-life of radio-labelled 25-hydroxyvitamin D3 in subjects receiving a high-fibre diet. British Journal of Nutrition 49, 213-216.

Beckman, M.J., Johnson, J.A., Goff, J.P., Reinhardt, T.A., Beitz, D.C. and Horst, R.L., 1995. The role of dietary calcium in the physiology of vitamin D toxicity: excess dietary vitamin D3 blunts parathyroid hormone induction of kidney 1-hydroxylase. Archives of Biochemistry & Biophysics 319, 535-539.

Binkley, N., Gemar, D., Engelke, J., Gangnon, R., Ramamurthy, R., Krueger, D. and Drezner, M.K., 2011. Evaluation of ergocalciferol or cholecalciferol dosing, 1,600 IU daily or 50,000 IU monthly in older adults. Journal of Clinical Endocrinology & Metabolism 96, 981-988.

Bischoff-Ferrari, H., 2009. Vitamin D: what is an adequate vitamin D level and how much supplementation is necessary? Best Practice & Research in Clinical Rheumatology 23, 789-795.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Staehelin, H.B., Orav, J.E., Stuck, A.E., Theiler, R., Wong, J.B., Egli, A., Kiel, D.P. and Henschkowski, J., 2009. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ 339, b3692.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Willett, W.C., Staehelin, H.B., Bazemore, M.G., Zee, R.Y. and Wong, J.B., 2004. Effect of Vitamin D on falls: a meta-analysis. JAMA 291, 1999-2006.

Bischoff-Ferrari, H.A., Giovannucci, E., Willett, W.C., Dietrich, T. and Dawson-Hughes, B., 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. American Journal of Clinical Nutrition 84, 18-28.

Bischoff-Ferrari, H.A., Willett, W.C., Wong, J.B., Giovannucci, E., Dietrich, T. and Dawson-Hughes, B., 2005. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 293, 2257-2264.

Bolland, M.J., Grey, A.B., Ames, R.W., Mason, B.H., Horne, A.M., Gamble, G.D. and Reid, I.R., 2006. Determinants of vitamin D status in older men living in a subtropical climate. Osteoporosis International 17, 1742-1748.

Boonen, S., Vanderschueren, D., Haentjens, P. and Lips, P., 2006. Calcium and vitamin D in the prevention and treatment of osteoporosis – a clinical update. Journal of Internal Medicine 259, 539-552.

Bouillon, R., 2011. Why modest but widespread improvement of the vitamin D status is the best strategy? Best Practice & Research Clinical Endocrinology & Metabolism 25, 693-702.

Burton, J.M., Kimball, S., Vieth, R., Bar-Or, A., Dosch, H.M., Cheung, R., Gagne, D., D'Souza, C., Ursell, M. and O'Connor, P., 2010. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis.[Erratum appears in Neurology. 2010 Aug 3;75(5):480].[Erratum appears in Neurology. 2010 Sep 14;75(11):1029 Note: Dosage error in article text]. Neurology 74, 1852-1859.

Buxton, I.L.O., 2006. Pharmacokinetics and pharmacodynamics: the dynamics of drug absorption, distribution, action, and elimination. In: L.L. Brunton, J.S. Lazo and K.L. Parker (eds.), Goodman & Gilman's the pharmacological basis of therapeutics. McGraw-Hill, New York, NY, USA.

Calvo, M.S., Whiting, S.J. and Barton, C.N., 2005. Vitamin D intake: a global perspective of current status. Journal of Nutrition 135, 310-316.

Cesur, Y., Caksen, H., Gundem, A., Kirimi, E. and Odabas, D., 2003. Comparison of low and high dose of vitamin D treatment in nutritional vitamin D deficiency rickets. Journal of Pediatric Endocrinology 16, 1105-1109.

Chel, V., Wijnhoven, H.A.H., Smit, J.H., Ooms, M. and Lips, P., 2008. Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. Osteoporosis International 19, 663-671.

Christakos, S., Dhawan, P., Liu, Y., Peng, X.R. and Porta, A., 2003. New insights into the mechanisms of vitamin D action. Journal of Cellular Biochemistry 88, 695-705.

Corless, D., Dawson, E., Fraser, F., Ellis, M., Evans, S.J., Perry, J.D., Reisner, C., Silver, C.P., Beer, M., Boucher, B.J. and Cohen, R.D., 1985. Do vitamin D supplements improve the physical capabilities of elderly hospital patients? Age & Ageing 14, 76-84.

Cranney, A., Horsley, T., O'Donnell, S., Weiler, H.A., Puil, L., Ooi, D.S., Atkinson, S.A., Ward, L.M., Moher, D., Hanley, D.A., Fang, M., Yazdi, F., Garritty, C., Sampson, M., Barrowman, N., Tsertsvadze, A. and Mamaladze, V., 2007. Effectiveness and Safety of Vitamin D in Relation to Bone Health. Evidence Report/Technology Assessment No. 158 (Prepared by the University of Ottawa Evidence-based Practice Center (UO-EPC) under Contract No. 290-02-0021. AHRQ Publication No. 07-E013), Rockville, MD, USA.

Cranney, A., Weiler, H.A., O'Donnell, S. and Puil, L., 2008. Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health. American Journal of Clinical Nutrition 88, 513S-519S.

Davies, M., Mawer, E.B., Hann, J.T., Stephens, W.P. and Taylor, J.L., 1985. Vitamin D prophylaxis in the elderly: a simple effective method suitable for large populations. Age & Ageing 14, 349-354.

Dawson-Hughes, B. and Harris, S.S., 2010. High-dose vitamin D supplementation: too much of a good thing? JAMA 303, 1861-1862.

Dawson-Hughes, B., Dallal, G.E., Krall, E.A., Harris, S., Sokoll, L.J. and Falconer, G., 1991. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. Annals of Internal Medicine 115, 505-512.

Dawson-Hughes, B., Heaney, R.P., Holick, M.F., Lips, P., Meunier, P.J. and Vieth, R., 2005. Estimates of optimal vitamin D status. Osteoporosis International 16, 713-716.

Dhesi, J.K., Jackson, S.H.D., Bearne, L.M., Moniz, C., Hurley, M.V., Swift, C.G. and Allain, T.J., 2004. Vitamin D supplementation improves neuromuscular function in older people who fall. Age & Ageing 33, 589-595.

Diamond, T.H., Ho, K.W., Rohl, P.G. and Meerkin, M., 2005. Annual intramuscular injection of a megadose of cholecalciferol for treatment of vitamin D deficiency: efficacy and safety data. Medical Journal of Australia 183, 10-12.

Ebeling, P. and Eisman, J.A., 2005. Recommendations from the vitamin D and calcium forum. Medicine Today 6, 43-50.

Flicker, L., MacInnis, R.J., Stein, M.S., Scherer, S.C., Mead, K.E., Nowson, C.A., Thomas, J., Lowndes, C., Hopper, J.L. and Wark, J.D., 2005. Should older people in residential care receive vitamin D to prevent falls? Results of a randomized trial. Journal of the American Geriatrics Society 53, 1881-1888.

Gallagher, J.C., Sai, A., Templin, T., 2nd and Smith, L., 2012. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. Annals of Internal Medicine 156, 425-437.

Gilchrest, B.A., 2008. Sun exposure and vitamin D sufficiency. American Journal of Clinical Nutrition 88, 570S-577S.

Giovannucci, E., 2011. Vitamin D, how much is enough and how much is too much? Public Health Nutrition 14, 740-741.

Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M., Newmark, H.L., Giovannucci, E., Wei, M. and Holick, M.F., 2007. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. American Journal of Preventive Medicine 32, 210-216.

Grimnes, G., Joakimsen, R., Figenschau, Y., Torjesen, P.A., Almas, B. and Jorde, R., 2012. The effect of high-dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass – a randomized controlled 1-year trial. Osteoporosis International 23, 201-211.

Grosvenor, M.B. and Smolin, L.A., 2002. Nutrition. From Science to Life. Harcourt College Publishers, Orlando, FL, USA.

Guillemant, S., Guillemant, J., Feteanu, D. and Sebag-Lanoe, R., 1989. Effect of vitamin D3 administration on serum 25-hydroxyvitamin D3, 1,25-dihydroxyvitamin D3 and osteocalcin in vitamin D-deficient elderly people. Journal of Steroid Biochemistry 33, 1155-1159.

Hackman, K.L., Gagnon, C., Briscoe, R.K., Lam, S., Anpalahan, M. and Ebeling, P.R., 2010. Efficacy and safety of oral continuous low-dose versus short-term high-dose vitamin D: a prospective randomised trial conducted in a clinical setting. Medical Journal of Australia 192, 686-689.

Haddad Jr., J.G. and Rojanasathit, S., 1976. Acute administration of 25-hydroxycholecalciferol in man. Journal of Clinical Endocrinology & Metabolism 42, 284-290.

Harwood, R.H., Sahota, O., Gaynor, K., Masud, T. and Hosking, D.J., 2004. A randomised, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: The Nottingham Neck of Femur (NONOF) Study. Age & Ageing 33, 45-51.

Hathcock, J.N., Shao, A., Vieth, R. and Heaney, R., 2007. Risk assessment for vitamin D. American Journal of Clinical Nutrition 85, 6-18.

Haworth, C.S., Jones, A.M., Adams, J.E., Selby, P.L. and Webb, A.K., 2004. Randomised double blind placebo controlled trial investigating the effect of calcium and vitamin D supplementation on bone mineral density and bone metabolism in adult patients with cystic fibrosis. Journal of Cystic Fibrosis 3, 233-236.

Heaney, R.P., 2005. The Vitamin D requirement in health and disease. Journal of Steroid Biochemistry & Molecular Biology 97, 13-19.

Heaney, R.P., Armas, L.A.G., Shary, J.R., Bell, N.H., Binkley, N. and Hollis, B.W., 2008. 25-Hydroxylation of vitamin D3: relation to circulating vitamin D3 under various input conditions. American Journal of Clinical Nutrition 87, 1738-1742.

Heaney, R.P., Davies, K.M., Chen, T.C., Holick, M.F. and Barger-Lux, M.J., 2003. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. American Journal of Clinical Nutrition 77, 204-210.

Heikinheimo, R.J., Haavisto, M.V., Harju, E.J., Inkovaara, J.A., Kaarela, R.H., Kolho, L.A. and Rajala, S.A., 1991. Serum vitamin D level after an annual intramuscular injection of ergocalciferol. Calcified Tissue International 49 Suppl, S87.

Heikinheimo, R.J., Inkovaara, J.A., Harju, E.J., Haavisto, M.V., Kaarela, R.H., Kataja, J.M., Kokko, A.M., Kolho, L.A. and Rajala, S.A., 1992. Annual injection of vitamin D and fractures of aged bones. Calcified Tissue International 51, 105-110.

Holick, M.F., 1995. Environmental factors that influence the cutaneous production of vitamin D. American Journal of Clinical Nutrition 61, 638S-645S.

Holick, M.F., 2001. Sunlight 'D'ilemma: risk of skin cancer or bone disease and muscle weakness. Lancet 357, 4-6.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Holick, M.F., 2008. Does vitamin D3 dosing schedule influence treatment efficacy in nursing home residents with vitamin D deficiency? Nature Clinical Practice Endocrinology & Metabolism 4, 656-657.

Holick, M.F., 2011. The IOM D-lemma. Public Health Nutrition 14, 939-941.

Holick, M.F., Biancuzzo, R.M., Chen, T.C., Klein, E.K., Young, A., Bibuld, D., Reitz, R., Salameh, W., Ameri, A. and Tannenbaum, A.D., 2008. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. Journal of Clinical Endocrinology & Metabolism 93, 677-681.

Hollis, B.W., 2000. Comparison of commercially available (125)I-based RIA methods for the determination of circulating 25-hydroxyvitamin D. Clinical Chemistry 46, 1657-1661.

Hollis, B.W., 2004. Editorial: The determination of circulating 25-hydroxyvitamin D: no easy task. Journal of Clinical Endocrinology & Metabolism 89, 3149-3151.

Honkanen, R., Alhava, E., Parviainen, M., Talasniemi, S. and Mönkkönen, R., 1990. The necessity and safety of calcium and vitamin D in the elderly. Journal of the American Geriatrics Society 38, 862-866.

Hyppönen, E. and Power, C., 2007. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. American Journal of Clinical Nutrition 85, 860-868.

Hypponen, E., Fararouei, M., Sovio, U., Hartikainen, A.-L., Pouta, A., Robertson, C., Whittaker, J.C. and Jarvelin, M.-R., 2011. High-dose vitamin D supplements are not associated with linear growth in a large Finnish cohort. Journal of Nutrition 141, 843-848.

Ilahi, M., Armas, L.A.G. and Heaney, R.P., 2008. Pharmacokinetics of a single, large dose of cholecalciferol. American Journal of Clinical Nutrition 87, 688-691.

Ish-Shalom, S., Segal, E., Salganik, T., Raz, B., Bromberg, I.L. and Vieth, R., 2008. Comparison of daily, weekly, and monthly vitamin D3 in ethanol dosing protocols for two months in elderly hip fracture patients. Journal of Clinical Endocrinology & Metabolism 93, 3430-3435.

Jackson, R.D., LaCroix, A.Z., Gass, M., Wallace, R.B., Robbins, J., Lewis, C.E., Bassford, T., Beresford, S.A.A., Black, H.R., Blanchette, P., Bonds, D.E., Brunner, R.L., Brzyski, R.G., Caan, B., Cauley, J.A., Chlebowski, R.T., Cummings, S.R., Granek, I., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Johnson, K.C., Judd, H., Kotchen, J.M., Kuller, L.H., Langer, R.D., Lasser, N.L., Limacher, M.C., Ludlam, S., Manson, J.E., Margolis, K.L., McGowan, J., Ockene, J.K., O'Sullivan, M.J., Phillips, L., Prentice, R.L., Sarto, G.E., Stefanick, M.L., Van Horn, L., Wactawski-Wende, J., Whitlock, E., Anderson, G.L., Assaf, A.R., Barad, D. and Women's Health Initiative, I., 2006. Calcium plus vitamin D supplementation and the risk of fractures. [Erratum, N Engl J Med 2006;354:1102]. New England Journal of Medicine 354, 669-683.

Johnson, K.R., Jobber, J. and Stonawski, B.J., 1980. Prophylactic vitamin D in the elderly. Age & Ageing 9, 121-127.

Jones, G., 2008. Pharmacokinetics of vitamin D toxicity. American Journal of Clinical Nutrition 88, 582S-586S.

Jorde, R., Sneve, M., Emaus, N., Figenschau, Y. and Grimnes, G., 2010a. Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromso study. European Journal of Nutrition 49, 401-407.

Jorde, R., Sneve, M., Figenschau, Y., Svartberg, J. and Waterloo, K., 2008. Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. Journal of Internal Medicine 264, 599-609.

Jorde, R., Sneve, M., Torjesen, P. and Figenschau, Y., 2010b. No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for 1 year. Journal of Internal Medicine 267, 462-472.

Jorde, R., Sneve, M., Torjesen, P.A., Figenschau, Y., Goransson, L.G. and Omdal, R., 2010c. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine 50, 175-180.

Kenny, A.M., Biskup, B., Robbins, B., Marcella, G. and Burleson, J.A., 2003. Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men. Journal of the American Geriatrics Society 51, 1762-1767.

Khaw, K.T., Scragg, R. and Murphy, S., 1994. Single-dose cholecalciferol suppresses the winter increase in parathyroid hormone concentrations in healthy older men and women: a randomized trial. American Journal of Clinical Nutrition 59, 1040-1044.

Kimball, S. and Vieth, R., 2008. Self-prescribed high-dose vitamin D3: effects on biochemical parameters in two men. Annals of Clinical Biochemistry 45, 106-110.

Kimball, S.M., Ursell, M.R., O'Connor, P. and Vieth, R., 2007. Safety of vitamin D3 in adults with multiple sclerosis. American Journal of Clinical Nutrition 86, 645-651.

Krause, R., Buhring, M., Hopfenmuller, W., Holick, M.F. and Sharma, A.M., 1998. Ultraviolet B and blood pressure. Lancet 352, 709-710.

Lanham-New, S.A., Buttriss, J.L., Miles, L.M., Ashwell, M., Berry, J.L., Boucher, B.J., Cashman, K.D., Cooper, C., Darling, A.L., Francis, R.M., Fraser, W.D., de Groot, C.P.G.M., Hypponen, E., Kiely, M., Lamberg-Allardt, C., Macdonald, H.M., Martineau, A.R., Masud, T., Mavroeidi, A., Nowson, C., Prentice, A., Stone, E.M., Reddy, S., Vieth, R. and Williams, C.M., 2011. Proceedings of the Rank Forum on Vitamin D. British Journal of Nutrition 105, 144-156.

Latham, N.K., Anderson, C.S. and Reid, I.R., 2003b. Effects of vitamin D supplementation on strength, physical performance, and falls in older persons: a systematic review. Journal of the American Geriatrics Society 51, 1219-1226.

Latham, N.K., Anderson, C.S., Lee, A., Bennett, D.A., Moseley, A., Cameron, I.D. and Fitness Collaborative, G., 2003a. A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). Journal of the American Geriatrics Society 51, 291-299.

Law, M., Withers, H., Morris, J. and Anderson, F., 2006. Vitamin D supplementation and the prevention of fractures and falls: results of a randomised trial in elderly people in residential accommodation. Age & Ageing 35, 482-486.

Liel, Y., Ulmer, E., Shary, J., Hollis, B.W. and Bell, N.H., 1988. Low circulating vitamin D in obesity. Calcified Tissue International 43, 199-201.

Lips, P., 2007. Vitamin D status and nutrition in Europe and Asia. Journal of Steroid Biochemistry & Molecular Biology 103, 620-625.

Lucas, J.A., Bolland, M.J., Grey, A.B., Ames, R.W., Mason, B.H., Horne, A.M., Gamble, G.D. and Reid, I.R., 2005. Determinants of vitamin D status in older women living in a subtropical climate. Osteoporosis International, 1641-1648.

Maalouf, J., Nabulsi, M., Vieth, R., Kimball, S., El-Rassi, R., Mahfoud, Z. and El-Hajj Fuleihan, G., 2008. Short- and long-term safety of weekly high-dose vitamin D3 supplementation in school children. Journal of Clinical Endocrinology & Metabolism 93, 2693-2701.

MacLaughlin, J. and Holick, M.F., 1985. Aging decreases the capacity of human skin to produce vitamin D3. Journal of Clinical Investigation 76, 1536-1538.

MacLennan, W.J. and Hamilton, J.C., 1977. Vitamin D supplements and 25-hydroxy vitamin D concentrations in the elderly. British Medical Journal 2, 859-861.

Mastaglia, S.R., Mautalen, C.A., Parisi, M.S. and Oliveri, B., 2006. Vitamin D2 dose required to rapidly increase 25OHD levels in osteoporotic women. European Journal of Clinical Nutrition 60, 681-687.

Mawer, E.B., Backhouse, J., Holman, C.A., Lumb, G.A. and Stanbury, S.W., 1972. The distribution and storage of vitamin D and its metabolites in human tissues. Clinical Science 43, 413-431.

McKenna, M.J., 1992. Differences in vitamin D status between countries in young adults and the elderly. American Journal of Medicine 93, 69-77.

Need, A.G., Morris, H.A., Horowitz, M. and Nordin, C., 1993. Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. American Journal of Clinical Nutrition 58, 882-885.

New Zealand Institute for Crop and Food Research Ltd., 2004. Foodfiles 2004®. New Zealand Institute for Crop and Food Research Ltd., Christchurch, New Zealand.

Nordin, B.E., Baker, M.R., Horsman, A. and Peacock, M., 1985. A prospective trial of the effect of vitamin D supplementation on metacarpal bone loss in elderly women. American Journal of Clinical Nutrition 42, 470-474.

Papadimitropoulos, E., Wells, G., Shea, B., Gillespie, W., Weaver, B., Zytaruk, N., Cranney, A., Adachi, J., Tugwell, P., Josse, R., Greenwood, C., Guyatt, G., Osteoporosis Methodology, G. and The Osteoporosis Research Advisory, G., 2002. Meta-analyses of therapies for postmenopausal osteoporosis. VIII: Meta-analysis of the efficacy of vitamin D treatment in preventing osteoporosis in postmenopausal women. Endocrine Reviews 23, 560-569.

Pekkarinen, T., Valimaki, V.-V., Aarum, S., Turpeinen, U., Hamalainen, E., Loyttyniemi, E. and Valimaki, M.J., 2010. The same annual dose of 292000 IU of vitamin D (cholecalciferol) on either daily or four monthly basis for elderly women: 1-year comparative study of the effects on serum 25(OH)D concentrations and renal function. Clinical Endocrinology 72, 455-461.

Premaor, M.O., Scalco, R., da Silva, M.J.S., Froehlich, P.E. and Furlanetto, T.W., 2008. The effect of a single dose versus a daily dose of cholecalciferol on the serum 25-hydroxycholecalciferol and parathyroid hormone levels in the elderly with secondary hyperparathyroidism living in a low-income housing unit. Journal of Bone & Mineral Metabolism 26, 603-608.

Prince, R.L., Austin, N., Devine, A., Dick, I.M., Bruce, D. and Zhu, K., 2008. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. Archives of Internal Medicine 168, 103-108.

Rapuri, P.B., Gallagher, J.C. and Haynatzki, G., 2004. Effect of vitamins D2 and D3 supplement use on serum 25OHD concentration in elderly women in summer and winter. Calcified Tissue International 74, 150-156.

Rockell, J.E.P., Skeaff, C.M., Williams, S.M. and Green, T.J., 2006. Serum 25-hydroxyvitamin D concentrations of New Zealanders aged 15 years and older. Osteoporosis International 17, 1382-1389.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 dietary reference intakes for calcium and vitamin D: what dietetics practitioners need to know. Journal of the American Dietetic Association 111, 524-527.

Ruston, D., Hoare, J., Henderson, L., Gregory, J., Bates, C.J., Prentice, A., Birch, M., Swan, G. and Farron, M., 2004. The national diet and nutrition survey: adults aged 19 to 64 years. Volume 4: Nutritional status (anthropometry and blood analytes), blood pressure and physical activity, TSO.

Sanders, K.M., Stuart, A.L., Williamson, E.J., Simpson, J.A., Kotowicz, M.A., Young, D. and Nicholson, G.C., 2010. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 303, 1815-1822.

Sato, Y., Iwamoto, J., Kanoko, T. and Satoh, K., 2005. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. Cerebrovascular Diseases 20, 187-192.

Schleithoff, S.S., Zittermann, A., Tenderich, G., Berthold, H.K., Stehle, P. and Koerfer, R., 2006. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. American Journal of Clinical Nutrition 83, 754-759.

Scragg, R., Khaw, K.T. and Murphy, S., 1995. Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults. European Journal of Clinical Nutrition 49, 640-646.

Smith, H., Anderson, F., Raphael, H., Maslin, P., Crozier, S. and Cooper, C., 2007. Effect of annual intramuscular vitamin D on fracture risk in elderly men and women – a population-based, randomized, double-blind, placebo-controlled trial. Rheumatology 46, 1852-1857.

Sneve, M., Figenschau, Y. and Jorde, R., 2008. Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects. European Journal of Endocrinology 159, 675-684.

Stamp, T.C., Haddad, J.G. and Twigg, C.A., 1977. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. Lancet 309, 1341-1343.

Suda, T., Ueno, Y., Fujii, K. and Shinki, T., 2003. Vitamin D and bone. Journal of Cellular Biochemistry 88, 259-266.

Tang, B.M.P., Eslick, G.D., Nowson, C., Smith, C. and Bensoussan, A., 2007. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet 370, 657-666.

Tjellesen, L., Hummer, L., Christiansen, C. and Rodbro, P., 1986. Different metabolism of vitamin D2/D3 in epileptic patients treated with phenobarbitone/phenytoin. Bone 7, 337-342.

Trang, H.M., Cole, D.E., Rubin, L.A., Pierratos, A., Siu, S. and Vieth, R., 1998. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. American Journal of Clinical Nutrition 68, 854-858.

Trivedi, D.P., Doll, R. and Khaw, K.T., 2003. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 326, 469.

Van Groningen, L., Opdenoordt, S., van Sorge, A., Telting, D., Giesen, A. and de Boer, H., 2010. Cholecalciferol loading dose guideline for vitamin D-deficient adults. European Journal of Endocrinology 162, 805-811.

Verschueren, S.M.P., Bogaerts, A., Delecluse, C., Claessens, A.L., Haentjens, P., Vanderschueren, D. and Boonen, S., 2011. The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: a 6-month randomized, controlled trial. Journal of Bone & Mineral Research 26, 42-49.

Vicchio, D., Yergey, A., Obrien, K., Allen, L., Ray, R. and Holick, M., 1993. Quantification and Kinetics of 25-Hydroxyvitamin-D3 by Isotope-Dilution Liquid-Chromatography Thermospray Mass-Spectrometry. Biological Mass Spectrometry 22, 53-58.

Vieth, R., 1999. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. American Journal of Clinical Nutrition 69, 842-856.

Vieth, R., 2004. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. Journal of Steroid Biochemistry & Molecular Biology 89-90, 575-579.

Vieth, R., 2007. Vitamin D toxicity, policy, and science. Journal of Bone & Mineral Research 22 Suppl 2, V64-68.

Vieth, R., 2008. Comment on Chel *et al.*: efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. Osteoporosis International 19, 721-722; author reply 723.

Vieth, R., 2011. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/l (30 ng/ml). Best Practice & Research Clinical Endocrinology & Metabolism 25, 681-691.

Vieth, R., Bischoff-Ferrari, H., Boucher, B.J., Dawson-Hughes, B., Garland, C.F., Heaney, R.P., Holick, M.F., Hollis, B.W., Lamberg-Allardt, C., McGrath, J.J., Norman, A.W., Scragg, R., Whiting, S.J., Willett, W.C. and Zittermann, A., 2007. The urgent need to recommend an intake of vitamin D that is effective. American Journal of Clinical Nutrition 85, 649-650.

Vieth, R., Chan, P.C. and MacFarlane, G.D., 2001. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. American Journal of Clinical Nutrition 73, 288-294.

Viljakainen, H.T., Palssa, A., Kärkkäinen, M., Jakobsen, J. and Lamberg-Allardt, C., 2006. How much vitamin D3 do the elderly need? Journal of the American College of Nutrition 25, 429-435.

Von Hurst, P.R., Stonehouse, W. and Coad, J., 2010. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial. British Journal of Nutrition 103, 549-555.

Von Restorff, C., Bischoff-Ferrari, H.A. and Theiler, R., 2009. High-dose oral vitamin D3 supplementation in rheumatology patients with severe vitamin D3 deficiency. Bone 45, 747-749.

Wasse, H., Huang, R., Long, Q., Singapuri, S., Raggi, P. and Tangpricha, V., 2012. Efficacy and safety of a short course of very-high-dose cholecalciferol in hemodialysis. American Journal of Clinical Nutrition 95, 522-528.

Webb, A.R., 2006. Who, what, where and when-influences on cutaneous vitamin D synthesis. Progress in Biophysics & Molecular Biology 92, 17-25.

Weisman, Y., Schen, R.J., Eisenberg, Z., Amarilio, N., Graff, E., Edelstein-Singer, M., Goldray, D. and Harell, A., 1986. Single oral high-dose vitamin D3 prophylaxis in the elderly. Journal of the American Geriatrics Society 34, 515-518.

Witham, M.D., Crighton, L.J., Gillespie, N.D., Struthers, A.D. and McMurdo, M.E.T., 2010a. The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure: a randomized controlled trial. Circulation: Heart Failure 3, 195-201.

Witham, M.D., Dove, F.J., Dryburgh, M., Sugden, J.A., Morris, A.D. and Struthers, A.D., 2010b. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 53, 2112-2119.

Wortsman, J., Matsuoka, L.Y., Chen, T.C., Lu, Z. and Holick, M.F., 2000. Decreased bioavailability of vitamin D in obesity. American Journal of Clinical Nutrition 72, 690-693.

Wu, F., Staykova, T., Horne, A., Clearwater, J., Ames, R., Mason, B., Orr-Walker, B., Gamble, G., Scott, M. and Reid, I., 2003. Efficacy of an oral, 10-day course of high-dose calciferol in correcting vitamin D deficiency. New Zealand Medical Journal 116, U536.

Zhu, K., Austin, N., Devine, A., Bruce, D. and Prince, R.L., 2010. A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. Journal of the American Geriatrics Society 58, 2063-2068.

Zhu, K., Devine, A., Dick, I.M., Wilson, S.G. and Prince, R.L., 2008. Effects of calcium and vitamin D supplementation on hip bone mineral density and calcium-related analytes in elderly ambulatory Australian women: a five-year randomized controlled trial. Journal of Clinical Endocrinology & Metabolism 93, 743-749.

Vitamin D and chronic disease

Key facts

- Mean global vitamin D status is close to the insufficiency range.
- Several experimental studies show that a complete or partial lack of vitamin D action results in premature death.
- Many cells (e.g. monocytes, dendritic cells, B-lymphocytes, colonocytes, smooth muscle cells, endothelial cells) also possess the 25(OH)D-1α-hydroxylase (CYP27B1) which is required for the ability to synthesize the active form $1,25(OH)_2D$, suggesting important regulatory properties of the active vitamin D metabolite on a cellular level.
- Some prospective cohort studies found a U-shaped association between circulating 25(OH)D levels and cancer morbidity and mortality with an increased risk at both, low and high levels.
- Adequately powered RCTs with all-cause mortality as primary endpoint are still needed.

Summary points

- There is likely evidence from prospective cohort studies and meta-analyses of randomized controlled trials where mortality was a secondary endpoint that adequate vitamin D status reduces all-cause mortality risk.
- Large randomized controlled trials with all-cause mortality or cause-specific mortality as primary endpoint are still lacking.
- Evidence from prospective cohort studies is accumulating that vitamin D deficiency increases the risk for cardiovascular diseases.
- Meta-analyses of cohort studies suggest that an increase of vitamin D reduces all-cause mortality in chronic kidney disease patients.
- For cancer mortality, available data does not allow final conclusions.

10. Vitamin D deficiency and premature death

A. Zittermann and S. Prokop
Clinic for Thoracic and Cardiovascular Surgery of the Heart and Diabetes Centre North Rhine-Westphalia, Georgstr. 11, 32545 Bad Oeynhausen, Germany; azittermann@hdz-nrw.de

Abstract

This chapter gives an overview of the current knowledge about vitamin D deficiency and premature death and discusses possible life-prolonging mechanisms of vitamin D. Globally, vitamin D status is low and the prevalence of vitamin D deficiency is common. Historically, rickets was associated with a high mortality risk in Europe and Northern America. In frail elderly people, both mortality risk and the prevalence of vitamin D deficiency increase exponentially. Likewise, prospective cohort studies indicate an elevated mortality risk in vitamin D deficient people in the general population. Results from two meta-analyses that have summarized available data from randomized studies where all-cause mortality was a secondary endpoint indicate a 6-7% reduction in all-cause mortality by vitamin D supplementation in frail elderly people. Studies with experimental animals lacking vitamin D action support the assumption that vitamin D deficiency results in premature death. Possible beneficial vitamin D actions include important regulatory properties of 1,25-dihydroxyvitamin D on a cellular level as well as higher telomerase activity, leading to maintained telomere length. Data from prospective cohort studies on cardiovascular mortality concur with those on all-cause mortality. For cancer mortality, data from epidemiological studies are less convincing and the evidence from randomized controlled trials is inconsistent. In conclusion, there is likely evidence that vitamin D reduces all-cause mortality in elderly people. However, adequately powered randomized controlled trials with all-cause mortality as primary endpoint are still needed.

Keywords: mortality, survival, premature death, cancer, cardiovascular disease, chronic kidney disease

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_10, © Wageningen Academic Publishers 2013

Abbreviation

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
CKD	Chronic kidney disease
CVD	Cardiovascular disease
HR	Hazard ratio
IOM	US Institute of Medicine
NHANES	National Health and Nutrition Examination Survey
RCT	Randomized controlled trial
RR	Relative risk
UVB	Ultraviolet B
VDR	Vitamin D receptor
WHI	Women's Health Initiative

10.1 Introduction

It has long been known that vitamin D is essential for human calcium and phosphorus homeostasis. These vitamin D effects are important for bone mineralization and bone health. This also explains why vitamin D deficiency causes rickets in infants and small children and osteomalacia in adults.

In recent decades, however, it became increasingly clear that besides its classical actions on mineral and bone metabolism, vitamin D also has a broad range of nonclassical actions in the human body. Experimental studies brought evidence forward that vitamin D is essential for muscle function, cardiovascular homeostasis, nervous function and the immune response. It is easy to imagine that disturbances in these biological systems lead to clinically relevant diseases, which may be life-threatening if the disturbances are severe. Low vitamin D status has indeed been associated with a high incidence of various chronic diseases such as CVD, various types of cancer, multiple sclerosis, and type 1 and 2 diabetes. This chapter gives an overview of the current knowledge about vitamin D deficiency and premature death. In addition, data on vitamin D deficiency and disease-specific mortality is presented. Since morbidity and mortality is sometimes closely linked with each other, e.g. in specific types of cancer, the association of vitamin D status with disease morbidity are also briefly elucidated. Possible life-prolonging mechanisms of vitamin D are discussed as well.

10.2 Human vitamin D status

Circulating 25(OH)D is the generally accepted indicator of human vitamin status. This parameter reflects the sum of vitamin D synthesis in the skin and oral vitamin D intake from dietary and supplemental sources. Recently, the IOM has stated that 25(OH)D levels below 30 nmol/l and between 30 and 50 nmol/l bear the risk of vitamin D deficiency and inadequacy, respectively. It

is noteworthy, that this statement is exclusively based on beneficial vitamin D effects on fracture risk. The IOM considers levels above 125 nmol/l as potentially harmful. While there is general agreement that both vitamin D deficiency and excess can be harmful, the cut-offs presented by the IOM have been criticized by many researchers. An international expert panel, for example, has recommended a target 25(OH)D range of at least 75 to 100 nmol/l and has proposed an upper safety limit for circulating 25(OH)D of 250 nmol/l (Souberbielle *et al.*, 2010), a value which is considerably higher than the upper safety limit of the IOM. Others consider values up to 372 nmol/l as safe (Holick, 2007). It is however also noteworthy that almost all classifications definitively consider circulating 25(OH)D levels <50 nmol/l and <25 nmol/l as inadequate and deficient, respectively. Table 10.1 presents a classification by the authors where the different stages of vitamin D status are associated to circulating 25(OH)D concentrations and are also related to possible biochemical and clinical alterations.

It is important to mention that the estimated mean global vitamin D level is 54 nmol/l (Hagenau *et al.*, 2009) and thus close to the insufficiency range. In apparently healthy adults in North America and Central Europe the prevalence of 25(OH)D levels in the deficiency range is 8% and 16%, respectively (Looker *et al.*, 2011; Zittermann, 2010), with a prevalence of up to 32% in African-Americans. Levels <25 nmol/l are also widespread in regions such as South Asia and the Middle East. In the Middle East, for example, 25(OH)D levels that are lying in the deficiency range have been reported in up to 60% of elderly people and up to 80% of young women. Older age, female sex, higher geographic latitude, winter season, darker skin pigmentation, less sunlight exposure, avoidance of vitamin D-rich foods such as fish, and absence of vitamin D fortification are the main factors that are significantly associated with lower 25(OH)D levels (Mithal *et al.*, 2009).

Table 10.1. Vitamin D status classified according to circulating 25(OH)D concentrations (Zittermann and Gummert, 2010).

Stage	25(OH)D (nmol/l)	Clinical/biochemical alterations
Deficiency	<25	Rickets, osteomalacia, myopathy, calcium malabsorption, severe hyperpara-thyreodism, low $1,25(OH)_2D$ levels, impaired immune and cardiovascular? function, death
Insufficiency	25-49.9	Reduced bone mineral density, impaired muscle function, elevated parathyroid hormone levels, slightly reduced $1,25(OH)_2D$ levels
Hypovitaminosis D/ suboptimal supply	50-74.9	Low bodily stores of vitamin D, slightly elevated parathyroid hormone levels
Adequacy	75-372	No disturbances of vitamin D-dependent functions
Intoxication	>372	Intestinal calcium hyperabsorption, hypercalcemia, soft tissue calcification, death

10.3 Vitamin D deficiency and all-cause mortality in the general population

10.3.1 Infants

In Europe and North America, rickets was historically associated with a high mortality risk. A century ago, for example, autopsies of infants who died at 18 months of age or less found histopathological evidence of rickets in 96% (214 of 221 cases) (Rajakumar, 2003). Unfortunately, randomized controlled trials highlighting the relation of rickets to mortality risk are scarce. In a recently published Indian study (Kumar *et al.*, 2011), 2079 low birthweight infants received 35 µg vitamin D or placebo weekly for 6 months. Primary endpoint was admission to hospital or death. Adjusted rate ratio for death or hospital admission was 0.93 (95% CI: 0.68, 1.29) and thus not different between the two study groups. However, it is noteworthy that at 6 months only 57% of infants in the vitamin D group had 25(OH)D levels lying in the adequate range (>50 nmol/l), whereas in 27% of infants in the placebo group values were also lying in the adequate range. Thus, the study was probably underpowered to find statistically significant differences between the vitamin D and placebo group in admission to hospital or death.

10.3.2 General adult population

Mortality risk increases exponentially in frail elderly people. These people also have a high risk for deficient 25(OH)D levels (Zittermann *et al.*, 2009). A recent prospective cohort study has demonstrated that deficient vitamin D status is independently associated with an elevated mortality risk in frail elderly individuals (Pilz *et al.*, 2012). Unfortunately, randomized controlled trials examining the effect of vitamin D on all-cause mortality as primary endpoint are completely lacking in the adult population. Nevertheless, results from two meta-analyses that have summarized available data from randomized studies where all-cause mortality was a secondary endpoint are available. The meta-analysis by Autier and Gandini (2007) included 18 RCTs with an overall sample of 57,113 individuals. The majority of these RCTs were originally undertaken to determine the effect of a vitamin D supplement on fracture risk in frail elderly people. Daily vitamin D intake varied from 300 to 2,000 IU yielding in a mean daily vitamin D dose of 528 IU. A total of 4,777 deaths occurred during a mean follow-up of 5.7 years. Combined estimates resulted in a decreased risk of total mortality of 7% (HR=0.93, 95% CI: 0.87, 0.99) in the vitamin D group during follow-up. In trials for which baseline 25(OH)D concentrations were available, covering 15,979 participants, values ranged from 22.0 to 47.0 nmol/l at baseline and increased to 62.0-105.0 nmol/l in vitamin D-supplemented individuals.

A more recent meta-analysis of the Cochrane Library by Bjelakovic *et al.* (2011) compromised of 50 randomized trials with 94,148 participants comparing a randomly assigned vitamin D supplement to a placebo- or non-interventional group. The vast majority of the trial population consisted of women older than 70 years. The Cochrane Review found that a supplementation with vitamin D3 decreases mortality significantly by about 6% (RR=0.94, 95% CI: 0.91, 0.98; 74,789 participants, 32 trials) whereas vitamin D2, alfacalcidol, or calcitriol ($1,25(OH)_2D3$) did not (Bjelakovic *et al.*, 2011). Alfacalcidol and calcitriol increased the risk of hypercalcaemia

(RR=3.18, 95% CI: 1.17, 8.68). Adverse effects such as renal stone formations were also identified in case vitamin D supplements were given in combination with calcium supplements (RR=1.17, 95% CI: 1.02, 1.34). In some of these studies, renal stone formation can reliably be attributed to supplementary calcium and not to the relatively low amount of supplementary vitamin D. Nevertheless, it cannot be definitely ruled out that frailty itself may increase the risk of renal stone formation after combined calcium and vitamin D supplementation. In physically active individuals similar amounts of calcium and vitamin D may lead to calcium excretion via sweat or calcium deposition in the skeleton.

Data on the association of circulating 25(OH)D serum concentrations with the mortality risk in the general adult population has been summarized in a recent meta-analysis of prospective cohort studies (Zittermann *et al.*, 2012). The meta-analysis consisted of two models: a nonparametric model and a parametric analysis to assess the dose-risk relation. The nonparametric model compared the highest level of 25(OH)D with the lowest level. Fourteen studies met the criteria for this model. The analysis included 62,584 individuals of whom 5,562 individuals died. The mean follow-up ranged from 1.3 to 27 years. In the nonparametric analysis, the summary estimate for highest compared with lowest categories of 25(OH)D showed a significant mortality risk reduction of 29% (95% CI: 0.50, 0.91). In the parametric model, eleven studies with a total of 59,231 individuals were analyzed. For this approach the lowest quantile (mean 25(OH)D value of 27.5 nmol/l) was used as a reference category. Findings from this analysis indicate a nonlinear decline in overall mortality with an individual RR of 0.86 (95% CI: 0.82, 0.91), 0.77 (95% CI: 0.70, 0.84) and 0.69 (95% CI: 0.60, 0.78) for increasing 25(OH)D serum levels of 12.5, 25.0 and 50.0 nmol/l, respectively. There was, however, no significant decrease in mortality when an increase of approximately 87.5 nmol/l above the reference category occurred. Data are in line with the suggestion of an exponential increase in morbidity and mortality at deficient 25(OH)D levels.

10.3.3 Critically ill patients

Vitamin D deficiency should also be a point of concern in critically ill patients. In an Australian study (Lee *et al.*, 2009), a total of 17% of intensive care unit patients had undetectable (<15 nmol/l) levels of 25(OH)D and additional 38% had levels below 30 nmol/l. The Simplified Acute Physiology Score II was highest in patients with deficient 25(OH)D levels, indicating more severe organ dysfunction than in patients with sufficient 25(OH)D levels. In addition, mortality rates were highest in vitamin D deficient patients. Similar results were reported from two hospitals in Boston (Braun *et al.*, 2011). Among a population of 1,325 critically ill patients, deficiency of 25(OH)D at the time of critical care initiation was a significant predictor of all-cause patient mortality.

10.3.4 Possible preventive mechanisms

Experimental studies support the assumption that vitamin D deficiency results in premature death. It has been described in several studies that a complete or partial lack of vitamin D action ($VDR^{-/-}$ mice and $CYP27B1^{-/-}$ mice) results in premature death. VDR mutant mice have growth

retardation, osteoporosis, kyphosis, skin thickening and wrinkling, alopecia, ectopic calcification, progressive loss of hearing and balance as well as a short life span (Tuohimaa, 2009). The active vitamin D metabolite, $1,25(OH)_2D$, functions through the VDR, which is expressed in almost all human cells and tissues. There are several known genes whose expression is influenced by the vitamin D-hormone $1,25(OH)_2D$ (Nagpal *et al.*, 2005). It is estimated that 3% of the genome are regulated directly or indirectly by vitamin D (Bouillon *et al.*, 2008). Furthermore, antiproliferative effects and effects on cell differentiation and angiogenesis are mechanisms discussed for beneficial effects of vitamin D. Many cells (e.g. monocytes, dendritic cells, B-lymphocytes, colonocytes, smooth muscle cells, endothelial cells) also possess the 25(OH)D-1α-hydroxylase (CYP27B1) which is required for the ability to convert 25(OH)D to the active form $1,25(OH)_2D$, suggesting important regulatory properties of $1,25(OH)_2D$ on a cellular level. A population-based cohort study in female twins highlighted a more general mechanism (Richards *et al.*, 2007). This study reported longer leukocyte telomere length with higher circulating 25(OH)D concentrations. Leukocyte telomere length is a predictor of aging-related disease and is positively related to longevity. The difference in leukocyte telomere length between the highest and lowest tertiles of circulating 25(OH)D (mean values: 41.0 and 124.3 nmol/l, respectively) was 107 base pairs, which is equivalent to 5 years of telomeric aging. A more recent study (Zhu *et al.*, 2011) has demonstrated that oral supplementation with 60,000 IU vitamin D3 per month (equivalent to approximately 2,000 IU per day) significantly increased peripheral blood monocyte cell telomerase activity in overweight African Americans. Telomerase is an essential enzyme for maintaining telomere length.

10.4 Vitamin D deficiency and cardiovascular mortality

Globally, CVD is the number one cause of mortality. In 2005, CVD was responsible for approximately 30% deaths worldwide. CVD includes various illnesses such as coronary heart disease, peripheral arterial diseases, cerebrovascular diseases such as stroke, and congestive heart failure (Zittermann and Gummert, 2010).

10.4.1 General adult population

Ecological studies have reported higher rates of coronary heart disease with increasing distance from the equator, a phenomenon that can be attributed to the higher prevalence of vitamin D deficiency in regions with less exposure to sunlight (Zittermann and Koerfer, 2008). Research on vitamin D for CVD has increased more rapidly in the recent past years than for any other disease. A substantial number of observational cohort studies are available and several possible mechanism by which vitamin D may affect the outcome of CVDs have been discussed (see below).

A meta-analysis of prospective cohort studies by Grandi *et al.*, (2010) evaluated the association of vitamin D status with cardiovascular morbidity and mortality. The analysis included four studies on cardiovascular incidence and 5 studies on cardiovascular mortality. Data were presented as HR for the lowest 25(OH)D quantile. The highest quantile served as reference category. For incidence

studies, the HR was 1.53 (95% CI: 1.22, 1.95) and for mortality studies the HR was 1.83 (1.19, 2.80). However, it was mentioned by the authors of that study themselves that heterogeneity and the small number of studies which could be included in the data analysis limits the interpretation and generalization of the results (Grandi *et al.*, 2010).

Meanwhile, various other prospective cohort studies evaluating the association between circulating 25(OH)D and cardiovascular mortality have been published. Results of 13 individual studies that were published until 2011 are illustrated in Figure 10.1. In summary, the data indicate an increased cardiovascular mortality risk if 25(OH)D levels are lying in the deficiency range.

In 2010, Wang *et al.* published a meta-analysis of RCTs on vitamin D and cardiovascular risk. All studies were performed in postmenopausal women or elderly people with preserved kidney function. The vitamin D dose ranged between 10 µg daily and 2.5 mg every 4 months, the latter being equivalent to approximately 20 µg vitamin D daily. The CVD risk was slightly (RR=0.90 and 0.83, respectively) but not significantly lower in the two studies testing solely the effect of vitamin D. In 1 of these 2 studies, CVD mortality was also assessed and was 0.84 (95% CI: 0.65, 1.10) in the supplementation group. In the 2 studies that used a combination of vitamin D and calcium, CVD risk was 1.21 and 1.04 in the supplementation group. The pooled CVD risk for the two vitamin D studies and the two vitamin D plus calcium studies was 0.90 (0.77, 1.05) and 1.04 (0.92, 1.18), respectively in the supplemented groups (Wang *et al.*, 2010). In total, results are in gross agreement with the observation that supplemental calcium increases CVD risk. It was concluded

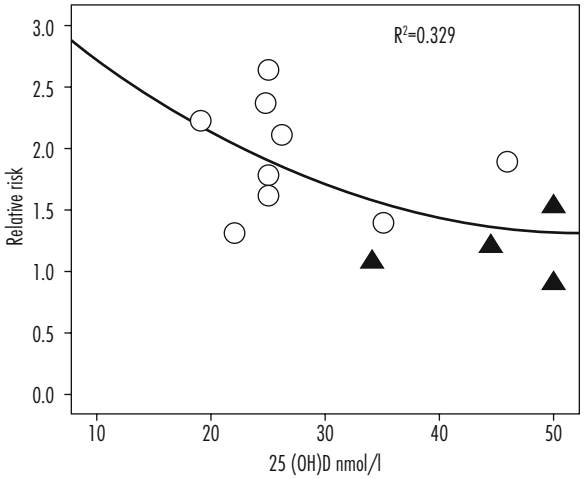

Figure 10.1. Relative risk of fatal cardiovascular events according to 25(OH)D levels; results from prospective cohort studies; data are adopted from Zittermann *et al.* (2012). Each point stands for a single study. Results represent the multivariable adjusted RR of a fatal cardiovascular event for the lowest 25(OH)D quantile compared to the respective highest 25(OH)D quantile. Circles indicate significant differences whereas triagles indicate no significant differences (95% CI includes 1).

by the authors of that meta-analysis that vitamin D supplements at moderate to high doses may reduce CVD risk. In a more recent study by Avenell *et al.* (2012), the incidence of vascular disease death in participants allocated to vitamin D compared to participants not allocated to vitamin D was very similar to the results of the meta-analysis by Wang *et al.* (2010) (HR=0.91; 95% CI: 0.79, 1.05). In *post hoc* analyses adjusted for compliance with vitamin D, vascular disease mortality (HR=0.76; 95% CI: 0.49, 1.40) also did not differ significantly in response to vitamin D, although all trends were accentuated compared to the intension to treat analysis. Again, the study was probably underpowered to find statistically significant differences between the vitamin D and placebo group.

10.4.2 Chronic kidney disease and cardiovascular mortality

CKD is an illness that predisposes patients to CVD. CKD is frequently associated with vascular calcification. Vascular calcification is an important predictor of cardiovascular and all-cause mortality in CKD patients (Rodriguez *et al.*, 2011). In parallel with the decline in kidney function, there is a progressive deterioration in mineral homeostasis including secondary hyperparathyroidism and low 25(OH)D and $1,25(OH)_2D$ levels.

Deficient levels of 25(OH)D are very prevalent in CKD (LaClair *et al.*, 2005; Naveneethan *et al.*, 2011). A recent meta-analysis of 10 prospective cohort studies (Pilz *et al.*, 2011a) with an overall sample of 6,853 patients found that an increase per 25 nmol/l of 25(OH)D reduces all cause-mortality by 14% (RR=0.86; 95%CI: 0.82, 0.91). There were no differences among patients with or without dialysis. Some more recently published studies (Pilz *et al.*, 2011b; Santoro *et al.*, 2011; Naveneethan *et al.*, 2011; Inaguma *et al.*, 2008) support the results of that earlier meta-analysis.

The 2009 'Kidney Disease: Improving Global Outcomes' clinical practice guidelines (KDIGO, 2009) include a weak recommendation to test for and treat vitamin D deficiency in patients with CKD stages 3-5D. However, the recommendations are based on the treatment strategies for the general population and detailed information about the amount and form of vitamin D supplementation is missing.

10.4.3 Possible preventive mechanisms of vitamin D against cardiovascular disease

There is experimental and clinical evidence for a cardio-protective role of vitamin D through anti-hypertensive, anti-inflammatory and anti-diabetic effects and through the control of parathyroid hormone and the renin-angiotensin aldosterone system (Pilz *et al.*, 2011c). Vitamin D also regulates intracellular calcium metabolism (Santillan and Boland, 1998), which plays a pivotal role in cardiac muscle contraction. In experimental studies, VDR knockout mice develop myocardial hypertrophy, arterial hypertension, and increased blood clotting tendency, nephropathy, and overexpression of the renin-angiotensin-aldosterone system (Bouillon *et al.*, 2008). Deletion of the cardiomyocyte-specific VDR gene results in a reduction in end-diastolic and end-systolic volume and increased atrial natriuretic peptide expression (Chen *et al.*, 2011).

10.5 Vitamin D deficiency and cancer mortality

10.5.1 Ecological studies

In the early eighties, Garland and Garland (1980) hypothesized that the impact of UVB radiation due to geographical differences correlates with cancer mortality (Garland and Garland, 1980). Since then, a number of ecological studies have shown that UVB radiation is inversely associated with cancer incidence as well as cancer mortality (Boscoe and Schymura, 2006; Grant, 2002; Grant and Mohr, 2009).

Sunlight exposure is the major vitamin D source in humans. Therefore, the effect of UVB irradiation concerning vitamin D supply may explain the results of the ecological studies. Due to the study design, reliability of geological studies is however limited. Nevertheless, data are in line with a death certificate case-control study that analyzed the relation between breast, ovarian, colon and prostate cancers and residential and occupational exposure to sunlight. In this exploratory study, residential exposure to sunlight was significantly associated with a reduced mortality from breast (OR=0.74; 95% CI: 0.72, 0.76), ovarian (OR=0.84; 95% CI: 0.81, 0.88), prostate (OR=0.84; 90% CI: 0.87, 0.93), and colon cancer (OR=0.73; 95% CI: 0.71, 0.74). Sunlight from occupational exposure played a minor role for cancer mortality. Non-melanoma skin cancers served as a positive control. It was positively associated with both residential (OR=1.23; 95% CI: 1.14, 1.33) and occupational (OR=1.15; 95% CI: 1.00, 1.32) skin cancer deaths (Freedman *et al.*, 2002).

10.5.2 Prospective cohort studies

Since vitamin D status depends on multiple factors such as skin pigmentation, actual UVB exposure and oral vitamin D intake, Giovannucci *et al.*, (2006) calculated a score that reflects long term 25(OH)D level due to the aforementioned vitamin D sources. The score was implemented in the ongoing Health Professionals Follow-up Study. Among 47,800 men, cancer mortality increased significantly in relation to predicted low vitamin D levels, whereas an increment of 25 nmol/l was associated with a 29% reduction in total cancer-mortality (RR=0.71; 95% CI: 0.60, 0.83) and 45% reduction in digestive-system cancer (RR=0.55, 95% CI: 0.41, 0.74).

The NHANES III was one of the first prospective studies that examined the relationship of low serum vitamin D levels and cancer mortality. A total number of 16,819 male and female participants were followed up from 1988-1994 through 2000. Among the study participants, 536 cancer deaths were identified during 146,578 person-years of observation. Because season and latitude are related to serum 25(OH)D levels and these parameters were linked in the dataset, the opportunity to assess each variable independent of the other was limited. Therefore, two subgroups were built 'winter/lower latitude' and 'summer/higher latitude.' However, season and latitude were unrelated to cancer mortality. High 25(OH)D levels were also not associated with a decline in total cancer mortality risk, but were significantly associated with colorectal cancer risk. In detail, 25(OH)D levels of 80 nmol/l or higher were associated with a decreased risk by 72% (HR=0.28, 95% CI: 0.11, 0.68; P=0.02) compared to levels less than 50 nmol/l (Freedman *et al.*, 2007). In an

additional analysis, the observation period of this trial was expanded over a period of six years from 2000 through 2006. In total, 884 cases of cancer deaths occurred during the entire follow-up. Again, overall cancer mortality was not related to vitamin D status. In addition, colorectal cancer mortality risk showed only a tendency for a decrease with higher 25(OH)D levels. However, the long follow-up of 16 years from initial 25(OH)D measurements is an important limitation of this study. Note that the correlation coefficient between 2 measurements of 25(OH)D taken 3 years apart is moderately high (0.70) (Platz *et al.*, 2004). On the one hand, this suggests that a single 25(OH)D measurement is a useful tool in epidemiologic studies with a medium follow-up. On the other hand, it also suggests that initial 25(OH)D measurement may not reliably reflect vitamin D status over a very long period. It is therefore important that a meta-analysis of eight prospective cohort studies including data from the Physician's Heath Study yielded a significant inverse association of colorectal cancer with 25(OH)D levels, regarding highest and lowest quartiles with pooled multivariate OR of 0.66 (95% CI: 0.54, 0.81) (Lee *et al.*, 2011). Another meta-analysis (Gandini *et al.*, 2011) identified 9 prospective studies with 2,630 study participants with data on serum 25(OH)D and colorectal cancer risk. The summary RR for a 25 nmol/l increase in serum 25(OH)D was 0.85 (95% CI: 0.79, 0.91). For breast cancer (6,175 cases in 10 studies) and prostate cancer (3,956 cases in 11 studies) no significant association with serum 25(OH)D was found. In a meta-analysis by Touvier *et al.* (2011), not only serum 25(OH)D levels but also dietary vitamin D intake was inversely associated with colorectal cancer risk. In detail, in 10 studies with daily vitamin D intakes ranging from 39 to 719 IU, RR of colorectal cancer decreased by 5% per 100 IU vitamin D intake, yielding a summary RR of 0.95 (95%CI: 0.93,0.98). In 6 studies, an increase in serum 25(OH)D by 6.2 nmol/l was associated with a RR of 0.96 (95%CI: 0.94,09.7).

The NHANES III study has also highlighted an important other issue concerning vitamin D and cancer risk: a subgroup analysis revealed a decreased cancer risk among women at higher altitude and who had blood drawings in summer and therefore mean 25(OH)D levels >100 nmol/l (RR=0.52; 95% CI: 0.25, 1.15). For men, however, there was an increased risk of overall cancer mortality with higher vitamin D levels (≥100 nmol/l) compared to the group with vitamin D levels lower 37.5 nmol/l (RR=1.85; 95% CI: 1.02, 3.35) concluding a sex specific impact of cancer incidence and mortality. Similar results were observed in a longitudinal nested case-control study of Nordic men (Ahn *et al.*, 2008). There was a U-shaped curve with both high (>80 nmol/l) and low (<19 nmol/l) levels of 25(OH)D associated with increased risk of prostate cancer. An increased risk at very high levels (≥100 nmol/l) was also noted for pancreatic cancer (Hetzlsouer, 2010). The causes for an increased cancer risk at 25(OH)D levels >100 nmol/l are unclear at present. Interestingly, experimental data demonstrate that high 25(OH)D levels can also be the result of low $1,25(OH)_2D$ levels (Vieth *et al.*, 1987). Thus, due to the lack of $1,25(OH)_2D$ measurements in the aforementioned studies it remains unclear whether excess vitamin D or deficient vitamin D action was responsible for the excess cancer risk in individuals with high 25(OH)D levels.

Currently, there is still inconsistency about the interpretation of epidemiologic data on vitamin D and cancer risk. The authors of the NHAMES III data recommend caution before encouraging vitamin D supplements for cancer preventing as the relationship of cancer and mortality might

be more difficult than suggested (Freedman *et al.*, 2010). These findings are in great contrast to the conclusion from Garland *et al.* in 2009 who estimated that raising the minimum year-around serum 25(OH)D level to 100-150 nmol/l has no unreasonable risk for the population, and would prevent approximately 58,000 new cases of breast cancer and 49,000 new cases of colorectal cancer each year in the United States and Canada (Garland *et al.*, 2009).

10.5.3 Randomized controlled trials

The present literature includes only very few randomized placebo-controlled trials that address cancer mortality in relation to vitamin D status. One of these RCTs is the three year long-term RECORD trial (Randomized Evaluation of Calcium Or vitamin D), involving 5,292 participants, mainly women, who were randomly assigned to receive either a daily supplement of 800 IU, 1000 mg calcium, both, or placebo. Cancer mortality did not differ significantly among the vitamin D and control group (HR=0.85; 95% CI: 0.68, 1.03). In a *post hoc* statistical analysis adjusting for compliance, trends for reduced mortality with vitamin D and increased mortality with calcium were accentuated, although all results remain non-significant (Avenell *et al.*, 2012).

In the WHI study, the largest clinical trial of vitamin D (400 IU/day) and calcium (1000 mg/day) so far, there was a nonsignificant (P=0.17) reduction of cancer mortality with supplementation during a mean follow-up of 7 years (Brunner *et al.*, 2011). Some studies have also analyzed cancer morbidity in subgroups of the WHI participants. Women concurrently treated with estrogen therapy had a decreased risk of colorectal cancer (Ding *et al.*, 2008). In another subgroup analysis of the WHI study (Bolland *et al.*, 2011) in 15,646 women who were not taking personnel calcium or vitamin D supplements at randomization, calcium plus vitamin D significantly reduced total, breast and invasive breast cancer by 14-20%, respectively, and nonsignificantly reduced the risk of colorectal cancer by 17%. In a 4-year randomized double-blind, placebo-controlled trial 1,179 community dwelling women received 1,400-1,500 mg supplemental calcium alone, supplemental calcium plus 1,100 IU vitamin D or placebo daily. The RR of incident cancer in the calcium plus vitamin D and calcium alone groups were 0.402 and 0.532, respectively. When analysis was confined to cancers diagnosed after the first 12 months, RR for the calcium plus vitamin D group fell to 0.232 (95% CI: 0.09, 0.60) but did not change significantly for the calcium alone group.

It is noteworthy that hitherto no data are available for men. Since observational studies found sex-specific differences in cancer mortality, randomized trials including an adequate number of men are needed.

10.5.4 Possible anticancer actions of vitamin D

Several anticancer actions have been attributed to the vitamin D hormone $1,25(OH)_2D$. These actions include anti-proliferative properties. The molecular mechanisms by which $1,25(OH)_2D$ may mediate anti-cancer effects have been shown to involve multiple pathways and in some cases be cell-type specific. Nevertheless, in most cell types that express functional VDR, exposure to $1,25(OH)_2D$ results in the accumulation of cells in the G0/G1 phase of the cell cycle. In breast,

colon, and prostate cancer cells, it has been demonstrated that $1,25(OH)_2D$ has the ability to induce apoptosis. One mechanism of apoptosis induction is the down regulating of mitogenic pathways, such as those induced by insulin-like growth factors. Additionally, $1,25(OH)_2D$ sensitizes cancer cells to different cytotoxic substances and radiation. Both, *in vitro* and *in vivo* experiments showed that $1,25(OH)_2D$ can also modulate angiogenesis, a key step in continues tumor growth and progression. In addition to the activation of genomic pathways, $1,25(OH)_2D$ also triggers rapid, non-genomic responses that activate a transmembrane signaling cascade. $1,25(OH)_2D$ also has anti-inflammatory properties. Since inflammation contributes to the development and progression of many cancers, the suppressive vitamin D effects on inflammatory processes might contribute to its beneficial effects in multiple cancers (Vanoirbeek *et al.*, 2011).

10.6 Conclusion

In adults, there is likely evidence from prospective cohort studies and especially from meta-analyses of RCTs where mortality was a secondary endpoint that vitamin D deficiency is associated with an elevated mortality risk. A similar statement concerning vitamin D and mortality risk has recently been made by the German Nutrition Society (DGE, 2012).

Data on cardiovascular mortality concur with results on all-cause mortality. Prospective cohort studies indicate that the risk of fatal events is highest when 25(OH)D levels are lying in the deficiency range. Although first RCTs indicate that risk reduction is probably small, the effects on the population's morbidity and life expectancy may be profound due to the large number of patients who are affected by CVD.

For cancer mortality, data from epidemiological studies are less convincing and the evidence from RCTs is inconsistent. There is also an urgent need to clarify the etiology of the U-shaped association between circulating 25(OH)D levels and cancer morbidity and mortality in some prospective cohort studies.

In summary, adequately powered RCTs with all-cause mortality as primary endpoint are still needed.

References

Ahn, J., Peters, U., Albanes, D., Purdue, M. P., Abnet, C.C., Chatterjee, N., Horst, R.L., Hollis, B.W., Huang, W.-Y., Shikany, J.M., Hayes, R.B., and the prostate, lung, colorectal, and ovarian cancer screening trial project team, 2008. Serum vitamin D concentration and prostate cancer risk: a nested case-control study. National Cancer Institute 100, 796-804.

Autier, P. and Gandini, S., 2007. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Archives of Internal Medicine 167, 1730-1737.

Avenell, A., Maclennan, G.S., Jenkinson, D.J., Mcpherson, G.C., Mcdonald, A.M., Pant, P.R., Grant, A.M., Campbell, M.K., Anderson, F.H., Cooper, C., Francis, R.M., Gillespie, W.J., Robinson, C.M., Torgerson, D.J., Wallace, W.A. and RECORD Trial Group, 2012. Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D3 and/or calcium (RECORD trial). Journal of Clinical Endocrinology & Metabolism 97, 614-622.

Bjelakovic, G.L., Nikolova, D., Whitfield, K., Wetterslev, J., Simonetti, R.G., Bjelakovic, M. and Gluud, C., 2011. Vitamin D supplementation for prevention of mortality in adults (Review). Cochrane Database of Systematic Reviews 207.

Bolland, M.J., Grey, A., Gamble, G.D. and Reid, I.R., 2011. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. American Journal Clinical Nutrition 94, 1144-1149.

Boscoe, F.P. and Schymura, M.J., 2006. Solar ultraviolet-B exposure and cancer incidence and mortality in the United States, 1993-2002. BMC Cancer 6, 264.

Bouillon, R., Carmeliet, G., Verlinden, L., Van Etten, E., Verstuyf, A., Luderer, H.F., Lieben, L., Mathieu, C. and Demay, M., 2008. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocrine Reviews 29, 726-776.

Braun, A., Chang, D., Mahadevappa, K., Gibbons, F.K., Liu, Y., Giovannucci, E. and Christopher, K.B., 2011. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Critical Care Medicine 39, 671-677.

Brunner, R.L., Wactawski-Wende, J., Caan, B.J., Cochrane, B.B., Chlebowski, R.T., Gass, M.L.S., Jacobs, E.T., Lacroix, A.Z., Lane, D., Larson, J., Margolis, K.L., Millen, A.E., Sarto, G.E., Vitolins, M.Z. and Wallace, R.B., 2011. The effect of calcium plus vitamin D on risk for invasive cancer: results of the women's health initiative (WHI) calcium plus vitamin D randomized clinical trial. Nutrition and Cancer 63, 827-841.

Chen, S., Law, C.S., Grigsby, C.L., Olsen, K., Hong, T.-T., Zhang, Y., Yeghiazarians, Y. and Gardner, D.G., 2011. Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy/clinical perspective. Circulation 124, 1838-1847.

DGE (German Nutrition Society), 2012. Vitamin D und Prävention ausgewählter chronischer Krankheiten. Available at: http://www.dge.de/pdf/ws/DGE-Stellungnahme-VitD-111220.pdf.

Ding, E.L., Mehta, S., Fawzi, W.W. and Giovannucci, E.L., 2008. Interaction of estrogen therapy with calcium and vitamin D supplementation on colorectal cancer risk: reanalysis of women's health initiative randomized trial. International Journal of Cancer 122, 1690-1694.

Freedman, D.M., Dosemeci, M. and Mcglynn, K., 2002. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. Occupational and Environmental Medicine 59, 257-262.

Freedman, D.M., Looker, A.C., Abnet, C.C., Linet, M.S. and Graubard, B.I., 2010. Serum 25-hydroxyvitamin D and cancer mortality in the NHANES III study (1988-2006). Cancer Research 70, 8587-8597.

Freedman, D.M., Looker, A.C., Chang, S.C. and Graubard, B.I., 2007. Prospective study of serum vitamin D and cancer mortality in the United States. Journal of the National Cancer Institute 99, 1594-1602.

Gandini, S., Boniol, M., Haukka, J., Byrnes, G., Cox, B., Sneyd, M.J., Mullie, P. and Autier, P. 2011. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. International Journal of Cancer 128, 1414-1424.

Garland, C.F. and Garland, F.C., 1980. Do sunlight and vitamin D reduce the likelihood of colon cancer? International Journal of Epidemiology 9, 227-231.

Garland, C.F., Gorham, E.D., Mohr, S.B. and Garland, F.C., 2009. Vitamin D for Cancer Prevention: Global Perspective. Annals of Epidemiology 19, 468-483.

Giovannucci, E., Liu, Y. and Willett, W.C., 2006. Cancer incidence and mortality and vitamin D in black and white male health professionals. Cancer Epidemiology, Biomarkers and Prevention 15, 2467-2472.

Grandi, N.C., Breitling, L.P., Vossen, C.Y., Hahmann, H., Wusten, B., Marz, W., Rothenbacher, D. and Brenner, H., 2010. Serum vitamin D and risk of secondary cardiovascular disease events in patients with stable coronary heart disease. American Heart Journal 159, 1044-1051.

Grant, W.B. and Mohr, S.B., 2009. Ecological studies of ultraviolet B, vitamin D and cancer since 2000. Annals of Epidemiology 19, 446-454.

Grant, W.B., 2002. An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. Cancer 94, 272-281.

Hagenau, T., Vest, R., Gissel, T., Poulsen, C., Erlandsen, M., Mosekilde, L. and Vestergaard, P., 2009. Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. Osteoporosis International 20, 133-140.

Helzlsouer, K.J. and VDPP Steering Committee, 2010. Overview of the cohort consortium vitamin D pooling project of rarer cancers. American Journal of Epidemiology 172, 4-9.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Inaguma, D., Nagaya, H., Hara, K., Tatematsu, M., Shinjo, H., Suzuki, S., Mishima, T. and Kurata, K., 2008. Relationship between serum 1,25-dihydroxyvitamin D and mortality in patients with pre-dialysis chronic kidney disease. Clinical and Experimental Nephrology 12, 126-131.

KDIGO, 2009. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney International Suppl S1-130.

Kumar, G.T., Sachdev, H.S., Chellani, H., Rehman, A.M., Singh, V., Arora, H. and Filteau, S., 2011. Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. BMJ 342, D2975-D2975.

LaClair, R.E., Hellman, R.N., Karp, S.L., Kraus, M., Ofner, S., Li, Q., Graves, K.L. and Moe, S.M., 2005. Prevalence of calcidiol deficiency in CKD: a cross-sectional study across latitudes in the United States. American Journal of Kidney Diseases 45, 1026-1033.

Lee, J.E., Li, H., Chan, A.T., Hollis, B.W., Lee, I.-M., Stampfer, M.J., Wu, K., Giovannucci, E. and Ma, J., 2011. Circulating levels of vitamin D and colon and rectal cancer: the physicians' health study and a meta-analysis of prospective studies. Cancer Prevention Research 4, 735-743.

Lee, P., Eisman, J.A. and Center, J.R., 2009. Vitamin D deficiency in critically ill patients. New England Journal of Medicine 360, 1912-1914.

Looker, A.C., Johnson, C.L., Lacher, D.A., Pfeiffer, C.M., Schleicher, R.L. and Sempos, C.T., 2011. Vitamin D status: United States, 2001-2006. NCHS Data Brief, 1-8.

Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhardt, P., Dawson-Hughes, B., Eisman, J.A., El-Hajj Fuleihan, G., Josse, R.G., Lips, P. and Morales-Torres, J., 2009. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International 20, 1807-1820.

Nagpal, S., Na, S. and Rathnachalam, R. 2005. Noncalcemic actions of vitamin D receptor ligands. Endocrine Reviews 26, 662-687.

Navaneethan, S.D., Schold, J.D., Arrigain, S., Jolly, S.E., Jain, A., Schreiber Jr, M.J., Simon, J.F., Srinivas, T.R. and Nally Jr, J.V., 2011. Low 25-hydroxyvitamin D levels and mortality in non-dialysis-dependent CKD. American Journal of Kidney Diseases 58, 536-543.

Pilz, S., Dobnig, H., Tomaschitz, A., Kienreich, K., Meinitzer, A., Friedl, C., Wagner, D., Piswanger-Sölkner, C., März, W. and Fahrleitner-Pammer, A., 2012. Low 25-hydroxyvitamin D is associated with increased mortality in female nursing home residents. Journal of Clinical Endocrinology & Metabolism 97, E653-E657.

Pilz, S., Iodice, S., Zittermann, A., Grant, W.B. and Gandini, S., 2011a. Vitamin D status and mortality risk in CKD: a meta-analysis of prospective studies. American Journal Kidney Disease 58, 374-382.

Pilz, S., Tomaschitz, A., Friedl, C., Amrein, K., Drechsler, C., Ritz, E., Boehm, B.O., Grammer, T.B. and Marz, W., 2011b. Vitamin D status and mortality in chronic kidney disease. Nephrology Dialysis Transplantation 26, 3603-3609.

Pilz, S., Tomaschitz, A., Marz, W., Drechsler, C., Ritz, E., Zittermann, A., Cavalier, E., Pieber, T.R., Lappe, J.M., Grant, W.B., Holick, M.F. and Dekker, J.M., 2011c. Vitamin D, cardiovascular disease and mortality. Clinical Endocrinology (Oxf) 75, 575-584.

Platz, E.A., Leitzmann, M.F., Hollis, B.W., Willett, W.C. and Giovannucci, E., 2004. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes and Control 15, 255-265.

Rajakumar, K., 2003. Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. Pediatrics 112, e132-e135.

Richards, J.B., Valdes, A.M., Gardner, J.P., Paximadas, D., Kimura, M., Nessa, A., Lu, X., Surdulescu, G.L., Swaminathan, R., Spector, T.D. and Aviv, A., 2007. Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. American Journal Clinical Nutrition 86, 1420-1425.

Rodriguez, M., Martinez-Moreno, J.M., Rodríguez-Ortiz, M.E., Muñoz-Castañeda, J.R. and Almaden, Y., 2011. Vitamin D and vascular calcification in chronic kidney disease. Kidney and Blood Pressure Research 34, 261-268.

Santillán, G.E. and Boland, R.L., 1998. Studies suggesting the participation of protein kinase A in 1,25(OH)2-vitamin D3-dependent protein phosphorylation in cardiac muscle. Journal of Molecular and Cellular Cardiology 30, 225-233.

Santoro, D., Gitto, L., Ferraro, A., Satta, E., Savica, V. and Bellinghieri, G., 2011. Vitamin D status and mortality risk in patients with chronic kidney disease. Renal Failure 33, 184-191.

Souberbielle, J.C., Body, J.J., Lappe, J.M., Plebani, M., Shoenfeld, Y., Wang, T.J., Bischoff-Ferrari, H.A., Cavalier, E., Ebeling, P.R., Fardellone, P., Gandini, S., Gruson, D., Guerin, A.P., Heickendorff, L., Hollis, B.W., Ish-Shalom, S., Jean, G., Von Landenberg, P., Largura, A., Olsson, T., Pierrot-Deseilligny, C., Pilz, S., Tincani, A., Valcour, A. and Zittermann, A., 2010. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. Autoimmunity Review 9, 709-715.

Touvier, M., Chan, D.S.M., Lau, R., Aune, D., Vieira, R., Greenwood, D.C., Kampman, E., Riboli, E., Hercberg, S. and Norat, T., 2011. Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. Cancer Epidemiology, Biomarkers & Prevention 20, 1003-1016.

Tuohimaa, P., 2009. Vitamin D and aging. Journal of Steroid Biochemistry Molecular Biology 114, 78-84.

Vanoirbeek, E., Krishnan, A., Eelen, G., Verlinden, L., Bouillon, R., Feldman, D. and Verstuyf, A., 2011. The anti-cancer and anti-inflammatory actions of 1,25(OH)2D3. Best Practice & Research Clinical Endocrinology & Metabolism 25, 593-604.

Vieth, R., Fraser, D. and Kooh, S.W., 1987. Low dietary calcium reduces 25-hydroxycholecalciferol in plasma of rats. The Journal of Nutrition 117, 914-918.

Wang, L., Manson, J.E., Song, Y. and Sesso, H.D. 2010. Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. Annals of Internal Medicine 152, 315-323.

Zhu, H., Guo, D., Li, K., Pedersen-White, J., Stallmann-Jorgensen, I.S., Huang, Y., Parikh, S., Liu, K. and Dong, Y., 2011. Increased telomerase activity and vitamin D supplementation in overweight African Americans. International Journal of Obesity (Lond). 36, 805-809.

Zittermann, A. and Gummert, J.F., 2010. Sun, vitamin D, and cardiovascular disease. Journal of Photochemistry and Photobiology B: Biology 101, 124-129.

Zittermann, A. and Koerfer, R., 2008. Vitamin D in the prevention and treatment of coronary heart disease. Current Opinion in Clinical Nutrition & Metabolic Care 11, 752-757.

Zittermann, A., 2010. The estimated benefits of vitamin D for Germany. Molecular Nutrition & Food Research 54, 1164-1171.

Zittermann, A., Iodice, S., Pilz, S., Grant, W.B., Bagnardi, V. and Gandini, S., 2012. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. American Journal Clinical Nutrition 95, 91-100.

Zittermann, A., Von Helden, R., Grant, W., Kipshoven, C. and Ringe, J.D., 2009. An estimate of the survival benefit of improving vitamin D status in the adult German population. Dermatoendocrinology 1, 300-306.

Key facts

- Circulating vitamin D must be hydroxylated by 1a-hydroxylase in the kidney to become active.
- The activity of 1a-hydroxylase is reduced as kidney function declines.
- Reduced levels of active vitamin D leads to secondary hyperparathyroidism in patients with chronic kidney disease (CKD).
- Secondary hyperparathyroidism induces bone disturbances and increased fracture risk.
- Since the 1970s vitamin D analogs have been used for treatment of secondary hyperparathyroidism in patients with CKD.

Summary points

- With declining renal function an increased prevalence of disturbances in the mineral metabolism (secondary hyperparathyroidism) and bone (renal osteodystrophy) develops.
- Calcitriol and active vitamin D analogs are used for treatment of secondary hyperparathyroidism and bone disease in patients with CKD.
- High levels of calcium and phosphate are associated with extra-osseous calcification.
- New active vitamin D analogs with selective properties are developed in order to suppress secondary hyperparathyroidism and avoid the concomitant high calcium and phosphate levels.
- Patients treated with calcitriol or active vitamin D analogs seem to have an improved survival independent of the mineral metabolism, although interventional trials should confirm this survival advantage.

11. Vitamin D and hyperparathyroidism in patients with chronic kidney disease

D. Hansen
Department of Medicine, Roskilde Hospital, Koegevej 7-14, 4000 Roskilde, Denmark and Department of Nephrology, Rigshospitalet, Blegdamsvej 9, 2100 København Ø, Denmark;
dittehansen@dadlnet.dk

Abstract

Secondary hyperparathyroidism is a common clinical problem in patients with chronic kidney disease (CKD). It causes disturbances in bone metabolism and increased risk of bone fracture. Secondary hyperparathyroidism develops as a consequence of reduced renal phosphate excretion and thereby elevated phosphate levels, hypocalcaemia, increased fibroblast growth factor 23 levels and reduced levels of calcitriol due to decreased renal 1α-hydroxylation of vitamin D. Treatment with calcitriol or its analogs suppress the secondary hyperparathyroidism. But increasing doses of calcitriol induces elevated levels of calcium and phosphate. Elevated calcium and phosphate levels are associated with extra-vascular calcification, cardiovascular disease and increased mortality. Active vitamin D analogs have been developed in order to increase the therapeutic window for parathyroid hormone suppression, without increasing phosphate and calcium levels. Observational studies have demonstrated an improved survival in patients with CKD treated with calcitriol or active vitamin D analogs. Both observational and animal studies suggest an effect of vitamin D and vitamin D analogs through mechanisms that are independent of the mineral metabolism.

Keywords: mineral metabolism, renal osteodystrophy, mortality, cardiovascular disease

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_11, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
CaSR	Calcium-sensing receptor
CKD	Chronic kidney disease
FGF-23	Fibroblast growth factor 23
FGFR	Fibroblast growth factor 23 receptor
GFR	Glomerular filtration rate
PTH	Parathyroid hormone
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor

11.1 Vitamin D in patients with chronic kidney disease

Vitamin D deficiency is common in patients with CKD. The prevalence increases as the kidney function declines. In patients with CKD5 (Table 11.1) around 70% has 25(OH)D deficiency (25(OH)D <50 nmol/l) and around 70% has $1,25(OH)_2D$ deficiency ($1,25(OH)_2D$ <22 pg/ml) (Hansen, 2012; Levin *et al.*, 2007).

The low levels of 25(OH)D are due to many factors. This population has an older age and increased prevalence of diabetes compared to the general population, factors known to be associated with decreased levels of 25(OH)D. CKD is often a disarming disease and these patients may have the skin less exposed to the sun. A decreased vitamin D3 synthesis in the skin of the uremic patient has been shown. In order to avoid uremic symptoms, hyperphosphatemia and elevated potassium, patients with CKD are holding a restrictive diet which can exaggerate 25(OH)D deficiency. Proteinuria may decrease the level of VDBP and thereby the level of circulating 25(OH)D. In patients with disturbed renal function, the activity of 25-hydroxylase (CYP27A1 and CYP2R1) in the liver is decreased and may decrease 25-hydroxylation of vitamin D2 and D3. Furthermore,

Table 11.1. Stages of chronic kidney disease (National Kidney Foundation, 2002).

Stage	Description	GFR (ml/min/1.73 m^2)
CKD1	Kidney damage with normal or elevated GFR	≥90
CKD2	Kidney damage with mild decrease in GFR	60-89
CKD3	Moderate decrease in GFR	30-59
CKD4	Severe decrease in GFR	15-29
CKD5	Kidney failure	<15 or dialysis (CKD5D)

treatment with activated vitamin D (calcitriol or its analogs), has been shown to decrease the activity of 25-hydroxylase in the liver (Armas and Heaney, 2011).

The vitamin D deficiency in patients with reduced renal function differs from other populations, because the 1α-hydroxylation of 25(OH)D in the kidney is disturbed. Even patients with sufficient nutritional supply or production in the skin of vitamin D2 and vitamin D3, may still be deficient in circulating active $1,25(OH)_2D$.

As the kidney mass declines the amount of 1α-hydroxylase (CYP27B1) decrease, and as glomerular filtration rate declines the delivery of vitamin D to 1α-hydroxylation via glomerular filtration declines.

FGF-23 is a recently discovered hormone synthesised by the bone osteocytes. FGF-23 inhibits the renal phosphate reabsorption, inhibits the renal 1α-hydroxylase, and increases the activity of 24-hydroxylase (CYP24). FGF-23 increases as renal function declines. The deficiency of $1,25(OH)_2D$ may be induced by FGF-23, as FGF-23 begins to rise already in CKD3 (Isakova *et al.*, 2011).

Phosphate is retained due to decreased excretion with declining renal function, and has the potential to further decrease the activity of 1α-hydroxylase.

11.2 Secondary hyperparathyroidism in patients with chronic kidney disease

Secondary hyperparathyroidism occurs with increasing prevalence as kidney function declines. Secondary hyperparathyroidism develops due to disturbances in the mineral metabolism (Figure 11.1). First, phosphate levels increase due to decreased renal phosphate excretion. Phosphate increases the stability of PTH mRNA. Second, calcium levels are lowered due to hyperphosphatemia and low levels of vitamin D. Hypocalcaemia is an important regulator of PTH levels. A decrease in calcium levels inactivates the CaSR of the parathyroid cell and PTH secretion is increased. Hypocalcaemia retards the PTH degradation in the parathyroid glands and increases the parathyroid cell division. Third, the low levels of 25(OH)D and $1,25(OH)_2D$ decrease the vitamin D induced suppression of PTH synthesis. Fourth, uremia increases the stability of PTH mRNA. Fifth, the expression of the CaSR and the synthesis of the VDR declines as kidney function decreases. Finally, FGF-23 has been shown to suppress PTH secretion and parathyroid cell proliferation. However, in uremic animals a reduced expression of the FGFR and the obligate FGFR cofactor klotho has been demonstrated.

Chronic stimulation of the parathyroid gland leads to diffuse hyperplasia which may progress into nodular formation with autonomous monoclonal growth and PTH production. Monoclonal proliferation is present in more than 50% of parathyroid glands removed by parathyroidectomy in uremic patients (Cunningham *et al.*, 2011).

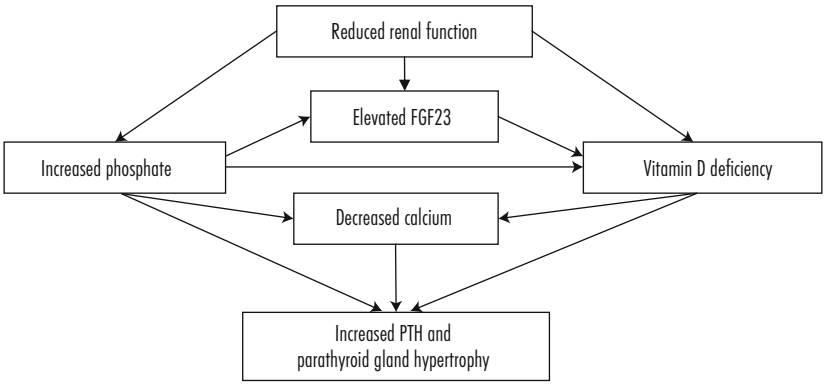

Figure 11.1. The pathogenesis of secondary hyperparathyroidism.

Several interventions are applied to prevent the development and treat the condition of secondary hyperparathyroidism.

Phosphate load is sought to be decreased by dietary advice. In dialysis patients phosphate levels may be reduced through increased dialysis dose, and calcium-load may be regulated by different concentrations of calcium in the dialysate. Phosphate binders are used to reduce the phosphate absorption from the intestine by binding the ingested phosphate. Two types of phosphate binders are available. Calcium-containing phosphate-binders increase calcium levels, while reducing phosphate levels. Non calcium-containing phosphate-binders reduce phosphate levels without an increase in calcium levels.

Vitamin D supplementation both as nutritional and active vitamin D analogs are used for suppression of PTH, and reduces hypocalcaemia (further discussed in the following section).

Calcimimetics increases the calcium sensibility of the CaSR in the parathyroid gland, thereby decreasing the PTH level. Calcimimetics are used in patients with concomitant hyperparathroidism and elevated ionised calcium levels.

Finally if hyperparathyroidism remains uncontrolled despite intensive medical strategies, parathyroidectomy may be needed.

11.3 Chronic kidney disease mineral and bone disorder

CKD mineral and bone disorder (Moe, 2006) is a systemic disorder of mineral and bone metabolism due to CKD manifested as either one or a combination of:

1. abnormalities of calcium, phosphate, PTH or vitamin D metabolism;
2. abnormalities of bone turn over, volume, mineralisation, linear growth or strength;
3. vascular or other soft tissue calcification.

The first has been described in the previous section.

Bone fracture occurs with a prevalence as high as 40-50% in a hemodialysis population. Furthermore, bone fracture in CKD patients is associated with a 2-2.5 times increased mortality compared to patients without CKD.

The abnormal bone histology in patients with CKD is termed renal osteodystrophy. The gold standard for diagnosis of renal osteodystrophy is a bone biopsy. Imaging methods and biochemical abnormalities as surrogate markers of histological bone abnormalities have been sought.

Measurement of PTH is at the moment the single most useful biomarker to predict bone histology. The three main conditions of renal osteodystrophy are (1) osteitis fibrosa cystica or high turnover bone disease associated with elevated PTH; (2) adynamic bone disease or low turnover bone disease associated with low PTH; (3) mixed disease.

Patients with CKD have increased mortality compared to the general population. In a hemodialysis population the annual mortality rate is around 20%. The risk of cardiovascular disease and cardiovascular mortality increase as kidney function declines.

The increased cardiovascular morbidity and mortality is only partially explained by traditional risk factors such as hypertension, diabetes and dyslipidemia.

The increased risk of cardiovascular disease and mortality is associated with disturbances in the mineral metabolism such as elevated calcium, phosphate and PTH levels. Calcium and phosphate stimulates vascular calcification *in vitro*. Observational studies have demonstrated an association between elevated calcium and phosphate levels and vascular calcification in coronary arteries. In CKD patients a high prevalence of calcification of the arterial smooth muscle layer (media calcification) is present, and associated with elevated calcium and phosphate levels. It gives rise to great concern that the bone minerals apparently deposits in the vasculature.

Like in the vasculature, elevated calcium and phosphate can induce soft tissue calcification in a wide variety of organs. Calcific uremic arteriolopathy with painful necrotic skin ulcerations is a particularly feared form of extra-osseous calcification. The exact interaction between calcium, phosphate and calcification inducers and inhibitors are under investigation.

Clinical guidelines propose target levels for ionised calcium, phosphate and PTH. These are based on the association between PTH and bone histology data, and based on cut-off values from epidemiologic studies describing the association between mineral metabolism and clinical outcomes such as bone fracture, cardiovascular disease and mortality. It is unknown whether reaching these targets improve clinical outcome, as no randomised clinical trial has addressed this question (Anonymous, 2009).

11.4 Treatment of vitamin D deficiency in patients with chronic kidney disease

In the 1970s 1α-hydroxylated metabolites of vitamin D were synthesised. Treatment with these active vitamin D analogs made it possible to bypass the problem concerning lack of 1α-hydroxylation in the kidney in patients with reduced renal function.

First, calcitriol ($1,25(OH)_2D3$) and alfacalcidol ($1α(OH)D3$) were provided to the uremic patients, in order to increase intestinal calcium absorption and thereby improve skeletal abnormalities. Later, the direct suppressive effect of $1,25(OH)_2D3$ on PTH synthesis in the parathyroid gland was discovered.

The ability of vitamin D analogs to suppress PTH decreases as CKD progress. This is caused by the progressive down regulation of the VDR and the CaSR in the parathyroid gland. Therefore increasing doses of vitamin D analogs are needed.

The use of vitamin D analogs is limited by their tendency to induce hypercalcemia and hyperphosphatemia. Vitamin D analogs increase the intestinal calcium and phosphate absorption, and enhance the PTH induced renal calcium reabsorption. Especially with the concomitant use of calcium-containing phosphate-binders, hypercalcemia is often an encountered problem.

New vitamin D analogs has been developed to increase the potency of PTH suppression without inducing hypercalcemia and hyperphosphatemia: doxercalciferol (1α-hydroxyvitamin D2), paricalcitol (19-nor-1α,25-dihydroxyvitamin D2), maxacalcitol (22-oxa-1α,25-dihydroxyvitamin D3) and falecalcitriol ($26,27\text{-}F_6$-1,25-dihydroxyvitamin D2).

The different analogs are briefly described in the following sections.

There are no clinical trials comparing the vitamin D analogs and their effect on clinical end points such as bone fracture, cardiovascular morbidity or mortality in CKD patients.

Alfacalcidol and doxercalciferol are classically considered pro-hormones to $1,25(OH)_2D3$ and $1,25(OH)_2D2$, which become active after 25-hydroxylation in the liver. However, alfacalcidol and doxercalciferol may have an intrinsic effect. In bovine parathyroid cells alfacalcidol and doxercalciferol suppressed PTH production and the PTH suppression persisted after 25-hydroxylase was blocked (Brown *et al.*, 2006).

11.4.1 Alfacalcidol

Alfacalcidol suppresses PTH with a concomitant increase in ionised calcium. The use of low-calcium in dialysis fluids makes it possible to induce a long-tem suppression of PTH by alfacalcidol in dialysis patients (Brandi, 2008). Long-term comparative studies of oral and intravenous alfacalcidol and calcitriol in hemodialysis patients found an equal suppression of PTH with

similar changes in calcium and phosphate levels (El-Reshaid *et al.*, 1997; Kiattisunthorn *et al.*, 2011). Alfacalcidol is widely used in Europe (Etalpha, Leo Pharma A/S, Ballerup, Denmark).

11.4.2 Paricalcitol

Paricalcitol lacks the exocyclic C19 in calcitriol, and has a vitamin D2 side chain. In animal studies paricalcitol was found to suppress PTH, while paricalcitol induced less increase in calcium and phosphate levels than calcitriol (Slatopolsky *et al.*, 1995). In hemodialysis patients a randomised study comparing calcitriol and paricalcitol found a faster PTH suppression in the paricalcitol treated patients, although no difference in the number of patients achieving PTH suppression after 32 weeks of treatment. However, the paricalcitol treated patients presented less episodes of persistent hypercalcemia (Sprague *et al.*, 2003). Paricalcitol was compared to alfacalcidol in a 16 week interventional study in hemodialysis patients, and both analogs suppressed PTH with similar increases in calcium and phosphate levels (Hansen *et al.*, 2011). Paricalcitol is widely used in America, Europe and Asia (Zemplar, Abbott Laboratories, Chicago, IL, USA).

11.4.3 Doxercalciferol

Doxercalciferol suppress PTH in CKD 3-5D in placebo controlled studies. The PTH suppression is at least partly induced by a concomitant increase in calcium levels (Coburn *et al.*, 2004; Frazao *et al.*, 2000; Tan, Jr. *et al.*, 1997). Doxercalciferol has been compared to calcitriol in a randomised 8 month trial in a paediatric peritoneal dialysis population. Both analogs suppressed PTH similar, with no differences in calcium or phosphate levels (Wesseling-Perry *et al.*, 2011). Doxercalciferol is available in the USA (Hectorol, Genzyme, Cambridge, MA, USA).

11.4.4 Maxacalcitol

A substitution of carbon 22 by an oxygen atom makes 22-oxacalcitriol differ from calcitriol. In 91 hemodialysis patients intravenous maxacalcitol and calcitriol was compared during 1 year in a randomised study. There was no difference between the number of patients reaching a PTH less than 150 pg/ml, and the final level of phosphate and calcium was similar between groups (Hayashi *et al.*, 2004). Intravenous maxacalcitol and oral calcitriol was compared in 46 hemodialysis patients in a randomised 24 week intervention study. Whereas calcitriol initially suppressed PTH faster accompanied by an increased calcium level, there was no difference in the final levels of PTH, phosphate and calcium, although hypercalcemia was more frequent in the calcitriol treated patients (Tamura *et al.*, 2005). Maxacalcitol is available in Japan (Oxarol, Chugai Pharmaceuticals CO., Tokyo, Japan).

11.4.5 Falecalcitriol

Falecalcitriol differs from calcitriol in carbon 26 and 27 where hydrogen atoms are substituted by fluoride atoms. Falecalcitriol was compared to alfacalcidol in a cross-over design in 25 hemodialysis patients. Oral doses were adjusted to maintain initial calcium levels during the

24 week treatment periods. Falecalcitriol tended to inhibit an increase in PTH, and the control of phosphate levels were better (Akiba *et al.*, 1998). When oral falecalcitriol was compared to intravenous calcitriol in 22 patients in a randomised cross-over trial, similar suppression of PTH was attained, with equal incidence of hypercalcemia and hyperphosphatemia and similar final calcium and phosphate levels (Ito *et al.*, 2009). Oral Falecalcitriol is available in Japan (Hornel, Taisho Pharmaceuticals Co., Tokyo, Japan, and Fulstan, Kissei Pharmaceuticals Co., Matsumoto, Japan).

11.4.6 Mechanisms for vitamin D analog selectivity

Continuous research tries to develop effective vitamin D analogs with selectivity for PTH suppression without calcium and phosphate level elevation. There are several possible interactions that may be modified in order to change the biological activity of the vitamin D analogs (Brown and Slatopolsky, 2008):

1. Binding to the VDBP. The VDBP carries 99% of calcitriol in the circulation. Binding to VDBP increases the circulating half life of and decreases the tissue accessibility of the vitamin D analog. Maxacalcitol has a 500 times lower affinity for VDBP than calcitriol, leading to faster clearance from the circulation, but a higher level in the intestine and parathyroid glands
2. Metabolism. Metabolism of the vitamin D analogs may influence on their activity. As discussed above alfacalcidol and doxercalciferol are classically considered to need 25-hydroxylation before they become active. However before they are 25-hydroxylated their affinity for VDBP is reduced, which may lead to increased accessibility into peripheral tissue as discussed in the proceeding section. The catabolism of vitamin D analogs by 24-hydroxylation attenuates the activity of the compounds. The 24-hydroxylase synthesis is induced by calcitriol and its analogs, and this induction may differ between the analogs. Modification of the vitamin D analog site chain affects their rate of catabolism, as Falecalcitriol which undergoes slower metabolic inactivation. Furthermore, as demonstrated for maxacalcitol, the catabolism may differ between cell-types. Maxacalcitol was faster degraded in keratinocytes than in parathyroid cells (Bikle *et al.*, 1995). The vitamin D analogs may be metabolised into active metabolites. Falecalcitriol are metabolised into an active 23-hydroxylated metabolite (Komuro *et al.*, 2003).
3. Interaction with VDR. After binding of the vitamin D analog to the VDR, the VDR undergoes conformational changes and interacts with the retinoid X receptor and co modulators and regulates gene transcription. The affinity for the VDR is important for the effect of the vitamin D analog. The binding of the vitamin D analog to the VDR may differ and induce differential conformational changes of the receptor and differential interaction with the co activators and co repressors of gene transcription. Differential recruitment of co activators has been described between maxacalcitol and calcitriol (Takeyama *et al.*, 1999).
4. Interactions with cell-surface receptor. It appears that calcitriol may act at the cell membrane through a rapid non-genomic pathway. The function of this pathway is still not fully understood. This pathway may induce rapid calcium and phosphate absorption in the enterocytes. It may be a potential target for new vitamin D analogs (Farach-Carson and Nemere, 2003).

5. Intracellular VDBPs. Intracellular proteins (HSC-70 and Bag-1) bind vitamin D analogs with high affinity and may thereby influence their activity and metabolism.

11.4.7 Native vitamin D

The native form of vitamin D; cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), are increasingly used in daily nephrology practice. The presence of the wide distribution of the VDR and 1α-hydroxylase in the body, makes a local synthesis of $1,25(OH)_2D$ possible, and native vitamin D may be needed for paracrine or autocrine functions in a wide range of cellular systems. Whether these local functions can be satisfied by supplementation with active vitamin D analogs are unknown. It may be necessary to substitute with both native and active vitamin D in CKD patients.

In parathyroid glands 1α-hydroxylase are present and can produce calcitriol (Ritter *et al.*, 2006). Treatment of low 25(OH)D levels has been shown to prevent the development and improve established hyperparathyroidism in CKD2-3, although the results in patients with more advanced kidney disease has not shown convincing evidence of PTH suppression (Al-Aly *et al.*, 2007; Shroff *et al.*, 2012; Zisman *et al.*, 2007).

11.5 Clinical outcomes and treatment with vitamin D in patients with chronic kidney disease

Multiple observational studies have shown an improved survival in CKD patients treated with activated vitamin D (Hansen, 2012). A survival advantage as high as 24% has been reported. An observational study even found an improved survival in patients treated with paricalcitol compared to calcitriol, although others found that this difference disappeared after adjustment for several variables.

The survival advantage linked to the use of active vitamin D has been shown to be present at different levels of calcium, phosphate and PTH, pointing towards an influence of vitamin D through other mechanisms than the mineral metabolism.

The survival benefit of vitamin D is further stressed by observational studies showing low levels of vitamin D both as 25(OH)D and $1,25(OH)_2D$ are associated with increased mortality in CKD patients (Pilz *et al.*, 2011).

No randomised controlled trial has addressed whether vitamin D treatment of any kind improve survival in CKD patients. Meta-analysis of vitamin D treatment and survival in predialysis and dialysis patients also found data too sparse to address this question (Palmer *et al.*, 2009a,b).

Possible mechanisms for a survival benefit in CKD patients treated with vitamin D are being explored. Especially the influence on renal function and cardiovascular disease in CKD patients

has been paid attention. Vitamin D may influence many cellular systems and organs in the CKD patients as well as in the general population (Holick, 2007), this is discussed in other chapters.

11.5.1 Renal function

Treatment with vitamin D analogs has been shown to decrease progression of renal insufficiency in observational studies (Kovesdy *et al.*, 2008; Shoben *et al.*, 2008)

Recently, the results of a multinational randomised trial found a decrease in albuminuria by paricalcitol compared to placebo in 281 type 2 diabetic patients with nephropathy (De Zeeuw *et al.*, 2010). Whether this decrease in albuminuria can be translated into a reduced progression in renal insufficiency is unknown.

Vitamin D down regulates the renin-angiotensin system by inhibiting the renin synthesis in animal studies (Li, 2003). Down regulation of the renin-angiotensin system by ACE-inhibitors and angiotensin receptor blockers has been shown to preserve kidney functions, therefore a renal protective function of vitamin D seams possible.

Vitamin D has anti-inflammatory properties (Mathieu and Adorini, 2002) and has been demonstrated to attenuate renal inflammation. This is a possible mechanism for preserving renal function.

11.5.2 Cardiovascular disease

The high mortality in CKD patients are at least in 50% caused by cardiovascular disease. Observational studies have demonstrated an association between cardiovascular mortality and both vitamin D deficiency and lack of treatment with vitamin D (Petchey *et al.*, 2011).

VDR knockout mice develops hypertension and left ventricular hypertrophy. Animal studies have pointed towards a reduction in left ventricular hypertrophy by vitamin D analogs. Like the reduction in albuminuria this may be mediated through an inhibition of the renin-angiotensin system. The results of a randomised placebo controlled trial in 227 CKD3-4 patients evaluating the change in left ventricular mass index during paricalcitol treatment are awaited.

Vascular calcification is common in CKD patients. High doses of vitamin D induce vascular calcification in experimental models. Vitamin D increases calcium and phosphate levels which are linked to increased vascular calcification. In both animal and observational studies a U-shaped curve between vitamin D dose and calcification was demonstrated pointing towards a therapeutic interval for vitamin D should be targeted.

References

Anonymous, 2009. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int. Suppl, S1-130.

Akiba, T., Marumo, F., Owada, A., Kurihara, S., Inoue, A., Chida, Y., Ando, R., Shinoda, T., Ishida, Y. and Ohashi, Y., 1998. Controlled trial of falecalcitriol versus alfacalcidol in suppression of parathyroid hormone in hemodialysis patients with secondary hyperparathyroidism. Am. J. Kidney Dis. 32, 238-246.

Al-Aly, Z., Qazi, R.A., Gonzalez, E.A., Zeringue, A. and Martin, K.J., 2007. Changes in serum 25-hydroxyvitamin D and plasma intact PTH levels following treatment with ergocalciferol in patients with CKD. Am. J. Kidney Dis. 50, 59-68.

Armas, L.A. and Heaney, R.P., 2011. Vitamin D: the iceberg nutrient. J. Ren Nutr., 21, 134-139.

Bikle, D.D., Abe-Hashimoto, J., Su, M.J., Felt, S., Gibson, D.F. and Pillai, S., 1995. 22-Oxa calcitriol is a less potent regulator of keratinocyte proliferation and differentiation due to decreased cellular uptake and enhanced catabolism. J. Invest Dermatol. 105, 693-698.

Brandi, L., 2008. 1alpha(OH)D3 One-alpha-hydroxy-cholecalciferol – an active vitamin D analog. Clinical studies on prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis. Dan. Med. Bull. 55, 186-210.

Brown, A.J., Ritter, C.S., Knutson, J.C. and Strugnell, S.A., 2006. The vitamin D prodrugs 1alpha(OH)D2, 1alpha(OH) D3 and BCI-210 suppress PTH secretion by bovine parathyroid cells. Nephrol. Dial. Transplant. 21, 644-650.

Brown, A.J. and Slatopolsky, E., 2008. Vitamin D analogs: therapeutic applications and mechanisms for selectivity. Mol. Aspects Med. 29, 433-452.

Coburn, J.W., Maung, H.M., Elangovan, L., Germain, M.J., Lindberg, J.S., Sprague, S.M., Williams, M.E. and Bishop, C.W., 2004. Doxercalciferol safely suppresses PTH levels in patients with secondary hyperparathyroidism associated with chronic kidney disease stages 3 and 4. Am. J. Kidney Dis. 43, 877-890.

Cunningham, J., Locatelli, F. and Rodriguez, M., 2011. Secondary hyperparathyroidism: pathogenesis, disease progression, and therapeutic options. Clin. J. Am. Soc. Nephrol. 6, 913-921.

De Zeeuw, D., Agarwal, R., Amdahl, M., Audhya, P., Coyne, D., Garimella, T., Parving, H.H., Pritchett, Y., Remuzzi, G., Ritz, E. and Andress, D., 2010. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet 376, 1543-1551.

El-Reshaid, K., el-Reshaid, W., Sugathan, T., al-Mohannadi, S. and Sivanandan, R., 1997. Comparison of the efficacy of two injectable forms of vitamin D3 and oral one-alpha in treatment of secondary hyperparathyroidism in patients on maintenance hemodialysis. Am. J. Nephrol. 17, 505-510.

Farach-Carson, M.C. and Nemere, I., 2003. Membrane receptors for vitamin D steroid hormones: potential new drug targets. Curr. Drug Targets. 4, 67-76.

Frazao, J.M., Elangovan, L., Maung, H.M., Chesney, R.W., Acchiardo, S.R., Bower, J.D., Kelley, B.J., Rodriguez, H.J., Norris, K.C., Robertson, J.A., Levine, B.S., Goodman, W.G., Gentile, D., Mazess, R.B., Kyllo, D.M., Douglass, L.L., Bishop, C.W. and Coburn, J.W., 2000. Intermittent doxercalciferol (1alpha-hydroxyvitamin D(2)) therapy for secondary hyperparathyroidism. Am. J. Kidney Dis. 36, 550-561.

Hansen, D., 2012. A randomised clinical study of alfacalcidol and paricalcitol. Dan. Med. J. 59, B4400.

Hansen, D., Rasmussen, K., Danielsen, H., Meyer-Hofmann, H., Bacevicius, E., Lauridsen, T.G., Madsen, J.K., Tougaard, B.G., Marckmann, P., Thye-Roenn, P., Nielsen, J.E., Kreiner, S. and Brandi, L., 2011. No difference between alfacalcidol and paricalcitol in the treatment of secondary hyperparathyroidism in hemodialysis patients: a randomized crossover trial. Kidney Int., 80, 841-850.

Hayashi, M., Tsuchiya, Y., Itaya, Y., Takenaka, T., Kobayashi, K., Yoshizawa, M., Nakamura, R., Monkawa, T. and Ichihara, A., 2004. Comparison of the effects of calcitriol and maxacalcitol on secondary hyperparathyroidism in patients on chronic haemodialysis: a randomized prospective multicentre trial. Nephrol. Dial. Transplant. 19, 2067-2073.

Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med. 357, 266-281.

Isakova, T., Wahl, P., Vargas, G.S., Gutierrez, O.M., Scialla, J., Xie, H., Appleby, D., Nessel, L., Bellovich, K., Chen, J., Hamm, L., Gadegbeku, C., Horwitz, E., Townsend, R.R., Anderson, C.A., Lash, J.P., Hsu, C.Y., Leonard, M.B. and Wolf, M., 2011. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. Kidney Int. 79, 1370-1378.

Ito, H., Ogata, H., Yamamoto, M., Takahashi, K., Shishido, K., Takahashi, J., Taguchi, S. and Kinugasa, E., 2009. Comparison of oral falecalcitriol and intravenous calcitriol in hemodialysis patients with secondary hyperparathyroidism: a randomized, crossover trial. Clin. Nephrol. 71, 660-668.

Kiattisunthorn, K., Wutyam, K., Indranoi, A. and Vasuvattakul, S., 2011. Randomized trial comparing pulse calcitriol and alfacalcidol for the treatment of secondary hyperparathyroidism in haemodialysis patients. Nephrology (Carlton) 16, 277-284.

Komuro, S., Sato, M. and Kanamaru, H., 2003. Disposition and metabolism of F6-1alpha,25(OH)2 vitamin D3 and 1alpha,25(OH)2 vitamin D3 in the parathyroid glands of rats dosed with tritium-labeled compounds. Drug Metab Dispos. 31, 973-978.

Kovesdy, C.P., Ahmadzadeh, S., Anderson, J.E. and Kalantar-Zadeh, K., 2008. Association of activated vitamin D treatment and mortality in chronic kidney disease. Arch. Intern. Med. 168, 397-403.

Levin, A., Bakris, G.L., Molitch, M., Smulders, M., Tian, J., Williams, L.A. and Andress, D.L., 2007. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. Kidney Int. 71, 31-38.

Li, Y.C., 2003. Vitamin D regulation of the renin-angiotensin system. J. Cell Biochem. 88: 327-331.

Mathieu, C. and Adorini, L., 2002. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. Trends Mol. Med. 8, 174-179.

Moe, S.M., 2006. Vascular calcification and renal osteodystrophy relationship in chronic kidney disease. Eur. J. Clin. Invest 36 Suppl 2, 51-62.

National Kidney Foundation, 2002. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am. J. Kidney Dis. 39, S1-266.

Palmer, S.C., McGregor, D.O., Craig, J.C., Elder, G., Macaskill, P. and Strippoli, G.F., 2009a. Vitamin D compounds for people with chronic kidney disease not requiring dialysis. Cochrane. Database. Syst. Rev., CD008175.

Palmer, S.C., McGregor, D.O., Craig, J.C., Elder, G., Macaskill, P. and Strippoli, G.F., 2009b. Vitamin D compounds for people with chronic kidney disease requiring dialysis. Cochrane. Database. Syst. Rev., CD005633.

Petchey, W.G., Johnson, D.W. and Isbel, N.M., 2011. Shining D' light on chronic kidney disease: mechanisms that may underpin the cardiovascular benefit of vitamin D. Nephrology (Carlton) 16, 351-367.

Pilz, S., Iodice, S., Zittermann, A., Grant, W.B. and Gandini, S., 2011. Vitamin D status and mortality risk in CKD: a meta-analysis of prospective studies. Am. J. Kidney Dis. 58, 374-382.

Ritter, C.S., Armbrecht, H.J., Slatopolsky, E. and Brown, A.J., 2006. 25-Hydroxyvitamin D(3) suppresses PTH synthesis and secretion by bovine parathyroid cells. Kidney Int. 70, 654-659.

Shoben, A.B., Rudser, K.D., de, B., I, Young, B. and Kestenbaum, B., 2008. Association of oral calcitriol with improved survival in nondialyzed CKD. J. Am. Soc. Nephrol. 19, 1613-1619.

Shroff, R., Wan, M., Gullett, A., Ledermann, S., Shute, R., Knott, C., Wells, D., Aitkenhead, H., Manickavasagar, B., Van't Hoff, W. and Rees, L., 2012. Ergocalciferol supplementation in children with CKD delays the onset of secondary hyperparathyroidism: a randomized trial. Clin. J. Am. Soc. Nephrol.

Slatopolsky, E., Finch, J., Ritter, C., Denda, M., Morrissey, J., Brown, A. and DeLuca, H., 1995. A new analog of calcitriol, 19-nor-1,25-(OH)2D2, suppresses parathyroid hormone secretion in uremic rats in the absence of hypercalcemia. Am. J. Kidney Dis. 26, 852-860.

Sprague, S.M., Llach, F., Amdahl, M., Taccetta, C. and Batlle, D., 2003. Paricalcitol versus calcitriol in the treatment of secondary hyperparathyroidism. Kidney Int. 63, 1483-1490.

Takeyama, K., Masuhiro, Y., Fuse, H., Endoh, H., Murayama, A., Kitanaka, S., Suzawa, M., Yanagisawa, J. and Kato, S., 1999. Selective interaction of vitamin D receptor with transcriptional coactivators by a vitamin D analog. Mol. Cell Biol. 19, 1049-1055.

Tamura, S., Ueki, K., Mashimo, K., Tsukada, Y., Naitoh, M., Abe, Y., Kawai, H., Tsuchida, A., Wakamatsu, R. and Nojima, Y., 2005. Comparison of the efficacy of an oral calcitriol pulse or intravenous 22-oxacalcitriol therapies in chronic hemodialysis patients. Clin. Exp. Nephrol. 9, 238-243.

Tan Jr., A.U., Levine, B.S., Mazess, R.B., Kyllo, D.M., Bishop, C.W., Knutson, J.C., Kleinman, K.S. and Coburn, J.W., 1997. Effective suppression of parathyroid hormone by 1 alpha-hydroxy-vitamin D2 in hemodialysis patients with moderate to severe secondary hyperparathyroidism. Kidney Int. 51, 317-323.

Wesseling-Perry, K., Pereira, R.C., Sahney, S., Gales, B., Wang, H.J., Elashoff, R., Juppner, H. and Salusky, I.B., 2011. Calcitriol and doxercalciferol are equivalent in controlling bone turnover, suppressing parathyroid hormone, and increasing fibroblast growth factor-23 in secondary hyperparathyroidism. Kidney Int. 79, 112-119.

Zisman, A.L., Hristova, M., Ho, L.T. and Sprague, S.M., 2007. Impact of ergocalciferol treatment of vitamin D deficiency on serum parathyroid hormone concentrations in chronic kidney disease. Am. J. Nephrol. 27, 36-43.

Key facts

- Over millennia, skin pigmentation adapts to where people live, dark enough to reduce free radical production and folate destruction, light enough to permit production of vitamin D.
- Sunlight reduces the risk of many types of disease primarily through production of vitamin D in the skin from the ultraviolet B (UVB) spectral region (290-315 nm).
- Ecological studies (population studies comparing geographical variation or seasonal variation of disease outcome with indices of risk factors) are often the first type of study to identify and quantify links to various risk-modifying factors.
- Vitamin D3 supplements are bioidentical to vitamin D produced by solar or artificial UVB.

Summary points

- Solar UVB and vitamin D reduce the risk of about 20 types of cancer.
- Solar UVB and vitamin D reduce the risk of several types of bacterial infectious diseases, including bacteremia, dental caries, pneumonia, sepsis, and tuberculosis.
- Solar UVB and vitamin D reduce the risk of several types of viral infectious diseases, including Epstein-Barr virus diseases (Hodgkin's lymphoma, multiple sclerosis, and infectious mononucleosis) and type A influenza.
- Solar UVB and vitamin D reduce the risk of several autoimmune diseases, including type 1 diabetes mellitus, multiple sclerosis, and rheumatoid arthritis.
- Hill's 1965 criteria for causality in a biological system can be used to assess whether links between solar UVB and/or vitamin D are causal. The criteria are strength of association, consistency, specificity (not applicable to vitamin D), temporality, biological gradient, plausibility (e.g. mechanisms), coherence, experiment (e.g. randomized controlled trial), and analogy. Later work added accounting for confounding factors and avoidance of bias.

12. Diseases affected by vitamin D: sun exposure

W.B. Grant
Sunlight, Nutrition and Health Research Center, P.O. Box 641603, San Francisco, CA 94164-1603, USA; wbgrant@infionline.net

Abstract

Solar ultraviolet B (UVB) irradiance, through production of vitamin D, is important in human health. In response to solar UV doses, skin pigmentation adapted over millennia to where people lived, changing from dark in the African plains to light in Northern Europe. Many ecological (geographical) studies have inversely correlated about 20 types of cancer with solar UVB doses. Observational studies along with empirical data from two randomized controlled trials (RCTs) largely support these ecological findings. Research has generally revealed the mechanisms whereby vitamin D can reduce cancer risk. By inducing cathelicidin and defensins, solar UVB reduces the risk of several bacterial and viral infections. Bacterial infections with strong evidence of UVB/vitamin D effects include dental caries, periodontal disease, pneumonia, sepsis, and tuberculosis. Viral infectious diseases with similar evidence include Epstein-Barr virus-linked diseases such as Hodgkin's lymphoma, multiple sclerosis, and infectious mononucleosis, as well as type A influenza. Solar UVB and vitamin D also reduce risk of other autoimmune diseases, including inflammatory bowel disease/Crohn's disease, rheumatoid arthritis, and type 1 diabetes mellitus. Good evidence for beneficial roles of UVB/vitamin D in reducing risk of cardiovascular disease (CVD) comes from observational studies of prediagnostic serum 25-hydroxyvitamin D and ecological studies of the seasonality of CVD incidence or mortality rates. That cold temperature also appears to be an important risk factor for CVD tends to somewhat cloud the seasonality studies. However, the same effect applies for type A influenza, yet two RCTs found beneficial effects of vitamin D supplementation. Although RCTs are often called for as definitive proof that vitamin D reduces the risk of various types of disease, the criteria for causality in a biological system that A.B. Hill established in 1965 can serve as an alternative approach.

Keywords: autoimmune disorders, cancer, cardiovascular disease, infections, ultraviolet B

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_12, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
CD	Crohn's disease
CVD	Cardiovascular disease
IBD	Inflammatory bowel disease
MS	Multiple sclerosis
NMSC	Nonmelanoma skin cancer
RA	Rheumatoid arthritis
RCT	Randomized controlled trial
SIR	Standardized incidence ratio
T1DM	Type 1 diabetes mellitus
UC	Ulcerative colitis
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B

12.1 Introduction

Over millennia, skin pigmentation varies throughout the world in response to ambient solar UV doses where people live. Those living in the tropical plains have dark skin, whereas those living in Northern Europe have pale skin. Skin pigmentation varies in response to two driving forces: (1) protection against the adverse effects of solar UV irradiance, free radical production and alteration of DNA, and destruction of folate; and (2) production of vitamin D (Jablonski and Chaplin, 2000, 2010). As people moved out of Africa to higher latitudes, those with lighter skin pigmentation had better bone development – improving the likelihood that the pelvic opening would be well formed to permit babies to exit the birth canal – as well as reduced risk of infectious diseases such as tuberculosis. Thus, we could expect that solar UVB doses, skin pigmentation, and time spent in the sun would have a major impact on general health and risk of many diseases. We also expect that health disparities between dark- and light-skinned people living outside the tropics could be due in part to differences in vitamin D production – which seems to be the case (Grant and Peiris, 2010, 2012).

The first disease linked to sunlight was rickets, for which Trousseau in France realized that lack of sunlight was a risk factor in 1861 (Rajakumar, 2003; Rajakumar *et al.*, 2007). However, not until 1922 did McCollum *et al.* demonstrate that vitamin D prevented or cured rickets. Rickets still occurs today, especially among breast-fed dark-skinned infants living in the midlatitudes (Rajakumar and Thomas, 2005).

Many more diseases are linked to low solar UVB doses and serum 25(OH)D concentrations. This chapter will highlight some of these diseases, presenting the evidence that both solar UVB irradiance and vitamin D reduce risk.

12.2 Diseases

12.2.1 Cancer

Many types of cancer are more common in regions with lower solar UVB doses. A paper published in 1937 noted that those who developed 'skin irritation' (probably NMSC and actinic keratoses) had a reduced risk of cancer incidence (Peller and Stephenson, 1937). A paper published in 1941 noted that US cancer mortality rates increase with latitude (Apperly, 1941). Not until 1980 did the brothers Cedric and Frank Garland propose the UVB-vitamin D-cancer hypothesis to explain the geographical variation of US colon cancer mortality rates (Garland and Garland, 1980). Higher dietary vitamin and calcium levels were associated with reduced risk of colorectal cancer (Garland *et al.*, 1985), and prediagnostic serum 25(OH)D concentration inversely correlated with colon cancer (Garland *et al.*, 1989). The geographical variations of mortality rates also supported the UVB-vitamin D-calcium hypothesis for breast (Garland *et al.*, 1990) and ovarian (Lefkowitz and Garland, 1994) cancer. Another group proposed that solar UVB reduced the risk of prostate cancer (Schwartz and Hulka, 1990). Pancreatic cancer increased with latitude in Japan (Kato *et al.*, 1985), and non-Hodgkin's lymphoma inversely correlated with solar UVB doses in contrast to the direct US association between solar UVB and melanoma and NMSC (Hartge *et al.*, 1996).

Although additional work supporting the UVB-vitamin D-cancer hypothesis emerged, the number of cancers with mortality rates inversely correlated with solar UVB doses did not reach 15 until 2002 (Grant, 2002). That study compared cancer mortality rates by state economic area with solar UVB doses for July 1992 (Leffell and Brash, 1996). US summertime solar UVB doses are highest in the Southwest, lowest in the Northeast (Leffell and Brash, 1996). The reasons include that the surface elevation is generally higher in the West and that the ozone layer is thinner as the westerly winds push the tropopause higher as the air masses prepare to cross the Rocky Mountains. Wintertime solar UVB doses vary directly with latitude (Grant, 2008b). That study led to other studies supporting the UVB-vitamin D-cancer hypothesis, such as an observational study from Harvard (Giovannucci *et al.*, 2006) and an RCT of vitamin D and calcium supplementation from Creighton University (Lappe *et al.*, 2007). That study received criticism for not having included other cancer risk-modifying factors, so the work was extended by including indices for alcohol consumption, ethnic background, poverty, smoking, and urban/rural residence, with similar results for solar UVB doses (Grant and Garland, 2006). A similar study was also published that year (Boscoe and Schymura, 2006). Several papers have reviewed ecological studies (Grant, 2012a,b; Grant and Mohr, 2009; Mohr, 2009). The most recent review 'consistently found strong inverse correlations with solar UVB for 15 types of cancer: bladder, breast, cervical, colon, endometrial, esophageal, gastric, lung, ovarian, pancreatic, rectal, renal, and vulvar cancer; and Hodgkin's and non-Hodgkin's lymphoma. Weaker evidence exists for nine other types of cancer: brain, gallbladder, laryngeal, oral/pharyngeal, prostate, and thyroid cancer; leukemia; melanoma; and multiple myeloma' (Grant, 2012a: 223).

Looking at cancer diagnosis or death related to incidence or death from NMSC is another way to show that solar UVB reduces cancer risk. A review of cohort studies reporting cancer incidence

rates with respect to NMSC diagnoses used this approach: 'For NMSC, relative risks for cervical, esophageal, gastric, and rectal cancer were significantly reduced; those for colon and gallbladder cancer were marginally insignificant, while those for female breast, laryngeal, ovarian, renal, and uterine corpus cancers were insignificantly reduced; relative risks for lip and salivary gland cancers and melanoma were significantly increased' (Grant, 2007a: 668). That same year, a record linkage study of cancers after diagnosis of NMSC in sunny countries (Australia, Singapore, and Spain) found significant inverse correlations with liver, pancreatic, and prostate cancer and found insignificant inverse correlations with bladder, colon, gastric, ovarian, rectal, and renal cancer (Tuohimaa *et al.*, 2007). In northern mid- and high-latitude countries, however, direct correlations emerged between diagnosis of NMSC and incidence of solid tumors. I attributed this finding to such factors as less body surface area exposed owing to colder weather (Grant, 2008a). Also, my ecological study of cancer mortality rate by province in Spain inversely correlated about 15 types of cancer with NMSC mortality rates (Grant, 2007b).

Using 1950-1964 cancer mortality rate data for California, Devesa *et al.* (1999) analyzed NMSC mortality rates as an index of solar UVB irradiance at the population level. NMSC rates were lower along the US West Coast than inland during 1950-1969, whereas mortality rates for internal cancers were often higher along the West Coast than inland, especially for males. Data for those older than 40 years in 5-year increments for the 19 state economic areas of California were obtained from the National Cancer Institute (http://ratecalc.cancer.gov/ratecalc/). Significant inverse correlations with NMSC mortality rate in multiple linear regression analyses emerged for 1950-1964 for eight types of cancer for males: bladder, brain, colon, gastric, prostate, and rectal cancer; multiple myeloma; and non-Hodgkin's lymphoma. No similar results emerged for females with respect to the other two UVB indices. Their NMSC mortality rates averaged 60% lower than those for males. Lung cancer mortality rates were directly correlated with three types of cancer for males: laryngeal, oral, and renal. No significant correlations with NMSC mortality rates appeared for later periods. Reasons for this finding may include reduced NMSC mortality rates, high immigration rates, and movement from rural to urban locations (Grant, 2012b).

As mentioned, most studies finding inverse correlations between solar UVB and cancer risk come from low and mid latitudes. Cancer survival after diagnosis is reportedly higher for summer or fall diagnosis than winter diagnosis for breast, colon, prostate cancer, and Hodgkin's lymphoma (Porojnicu *et al.*, 2007). Cancer incidence rates by occupation can show an effect of solar UVB in reducing cancer risk at higher latitudes. Data on cancer SIRs by sex and 54 occupation categories based on 1.4 million male and 1.36 million female cancer cases for 1961-2005 in the five Nordic countries undergird an ecological study of solar UVB's role in the risk of many types of cancer at high latitudes (Pukkala *et al.*, 2009). Lip cancer SIRs less lung cancer SIRs for men were the best index of solar UVB dose; these SIRs were inversely correlated with SIRs for both melanoma and NMSC. Lung cancer SIRs were used as the index of the effects of smoking. For men, the UVB index was significantly inversely correlated with 15 types of cancer: bladder, breast, colon, gallbladder, kidney, laryngeal, liver, lung, oral, pancreatic, pharyngeal, prostate, rectal, small intestine cancer, and melanoma. For women, the same UVB index was inversely correlated with

bladder, breast, colon, and rectal cancer. These results generally agree with findings from other studies (Grant, 2012c).

The inverse correlation between the UVB index in my study can be explained by how UVB irradiance protects against melanoma and basal cell carcinoma, the most common but least deadly of all skin cancers. Three ways exist: (1) increased pigmentation (tanning) and thickening of the stratum corneum; (2) vitamin D production (Field and Newton-Bishop, 2011); and (3) elastosis, or skin aging. Both solar UV and smoking lead to cross-linking of collagen in the skin. Skin elastosis reduces the risk of melanoma (Berwick *et al.*, 2005) and basal cell carcinoma (Walther *et al.*, 2004). Also, melanoma develops later in life for regions of the body chronically exposed to solar UV than to regions exposed only sporadically (Dal *et al.*, 2007). Elastosis might protect against melanoma because melanoma reaches out into the surrounding epidermis for fibroblasts (Flach *et al.*, 2011), which is harder to accomplish with elastosis.

The mechanisms whereby vitamin D reduces the risk of cancer are generally well known and include effects on cellular differentiation and proliferation, cellular adhesion, calcium absorption, metastasis, and angiogenesis (Fleet *et al.*, 2012; Garland *et al.*, 2009; Krishnan and Feldman, 2011; Lamprecht and Lipkin, 2003).

12.2.2 Infectious diseases

Solar UVB doses are associated with reduced risk of several bacterial and viral infectious diseases. Bacterial diseases include dental caries (Grant, 2011), periodontal disease (Grant and Boucher, 2010), pneumonia (Grant and Giovannucci, 2009), sepsis (Grant, 2010), and tuberculosis (Liu *et al.*, 2006, 2007). Viral infectious diseases included type A influenza (Cannell *et al.*, 2006, 2008), Epstein-Barr virus-related diseases such as Hodgkin's lymphoma (Douglas *et al.*, 1996; Hjalgrim *et al.*, 2007), infectious mononucleosis (Douglas *et al.*, 1996; Hjalgrim *et al.*, 2007), and MS (Grant and Holick, 2005; Orton *et al.*, 2011). Solar UVB reduces risk of these diseases through producing vitamin D and then inducing cathelicidin and defensins (Gombart, 2009). Cathelicidin has antimicrobial and antiendotoxin properties (Mookherjee *et al.*, 2007). Of these diseases, this chapter discusses two bacterial diseases, dental caries and sepsis, and two viral diseases, type A influenza and MS.

12.2.3 Dental caries

Solar UVB doses inversely correlated with dental caries among US male adolescents in the 1930s (East, 1939). However, evidence of a role of solar UVB in reducing risk of dental caries goes back to the Civil War, on the basis of a report that those trying to enlist in the Union Army from the Northeast had more missing teeth than those from as far south as Kentucky (Lewis, 1865). In 1928, an experiment with children found that oral vitamin D intake reduced the risk of dental caries (Mellanby and Pattison, 1928). Researchers of the era thought that vitamin D's role in increasing calcium absorption and putting it in the hard tissues explained the findings. However, 21st-century researchers found that cathelicidin killed the bacteria responsible for caries (Dale

and Fredericks, 2005). I published a unifying review of the literature in 2011 (Grant, 2011). A similar review was made for periodontal disease (Grant and Boucher, 2010), for which the role of solar UVB is less evident.

12.2.4 Type A influenza and pneumonia

Influenza is largely seasonal, with highest rates in winter, lowest rates in summer (Hope-Simpson, 1981). Cannell and colleagues hypothesized that the seasonality was due largely to annual variations in solar UVB doses and vitamin D production (Cannell *et al.*, 2006), with this hypothesis updated in 2008 (Cannell *et al.*, 2008). RCTs (Aloia and Li-Ng, 2007; Urashima *et al.*, 2010) and observational studies (Sabetta *et al.*, 2010) have supported this hypothesis. However, an opposing point of view is that influenza is largely seasonal because of changes in temperature and absolute humidity: the influenza virus survives longer outside the body when temperatures are cold and absolute humidity is low (Shaman *et al.*, 2010, 2011a,b). Both hypotheses seem to explain features of the annual cycle of influenza infections. Nature largely controls temperature and humidity; oral vitamin D intake can compensate for low solar UVB doses in winter.

One effect of influenza is rapid increase of proinflammatory cytokines, giving rise to a cytokine storm (Cheng *et al.*, 2011), which can disrupt the cells lining the lungs, thereby increasing the risk of bacterial or viral pneumonia. Most people who die soon after developing influenza die from pneumonia, approximately 10 days after the initial influenza infection. During the 1918-1919 pandemic, US rates of A/H1N1 influenza were much higher in the Northeast than in the South and West (Britten, 1932). An ecological study on case-fatality rates found that indices for summer and winter solar UVB doses explained about half of the variance (Grant and Giovannucci, 2009). A study in Philadelphia significantly inversely correlated solar UVB doses with infection by invasive pneumococcal disease, a common cause of community-acquired pneumonia (White *et al.*, 2009). An RCT found that vitamin D supplementation reduced the risk of pneumonia in children (Manaseki-Holland *et al.*, 2010).

12.2.5 Multiple sclerosis

Prevalence of MS increases with latitude (Kurtzke, 1993). However, prevalence in the Nordic countries, where more fish is consumed, is lower than in European countries just to the south (Kurtzke, 1993). The US prevalence of MS (on the basis of men enlisting in the armed forces during World War II and the Korean War (Wallin *et al.*, 2004)) shows a pronounced latitude dependence that indicates wintertime serum 25(OH)D concentrations rather than summertime concentrations (Grant and Holick, 2005). Other studies have also reported low solar UVB doses in winter as a risk factor (Orton *et al.*, 2011; van der Mei *et al.*, 2003). In the UK, seasonal UVB doses and infectious mononucleosis prevalence explained 72% of the variance of MS prevalence (Ramagopalan *et al.*, 2011).

12.2.6 Inflammatory bowel disease

IBD occurs in two forms: CD and UC. Two studies reported higher rates of IBD for higher latitudes than for lower latitudes, one in Europe (Shivananda *et al.*, 1996) and the other in France (Nerich *et al.*, 2006, 2010); one study also reported a latitudinal gradient for CD in Scotland (Armitage *et al.*, 2004). The geographical variation for CD in France is similar to that for breast cancer (Engel *et al.*, 2011; Grant, 2010) and other cancers (Grant, 2010); where this variation was linked to solar UVB doses and vitamin D production. However, the geographical variation of UC in France does not show a similar latitudinal gradient.

US rates for CD and UC are highest in the Northeast (Kappelman *et al.*, 2007; Sonnenberg, 2010). US summertime solar UVB doses are highest in the Southwest, lowest in the Northeast (Leffell and Brash, 1996). An ecological study using summertime UVB, wintertime UVB, smoking, and obesity analyzed the data, both prevalence and mortality rates by state, for CD, UC, and *Clostridium difficile* colitis in the US (Sonnenberg, 2010). For CD, summertime UVB (inverse association or risk reduction) and smoking (risk) were significantly correlated. For UC, UVB was significantly inversely correlated, but not as strongly as for DC. Smoking was correlated with *C. difficile* colitis (Grant, unpublished data). A study from India reported lower serum 25(OH)D levels in those with CD with lower sun exposure (Joseph *et al.*, 2009).

12.2.7 Type 1 diabetes mellitus

The seasonality of birth for those diagnosed with T1DM generally has higher rates in summer and lower rates in winter than all birth rates in the population. Studies have reported this trend for several countries, including New Zealand (Willis *et al.*, 2002), the United States (peak from March to June) (Kahn *et al.*, 2009), but not most European countries (McKinney, 2001). Higher rates in summer suggest that low serum 25(OH)D levels in late winter or early spring are a risk factor for T1DM. The 3- to 5-month lag between minimum serum 25(OH)D levels and spring or summertime birth corresponds to the effect of low serum 25(OH)D having greatest impact in the fourth to sixth month of gestation. This time may be when the pancreas develops. Pancreas A cell density was greatest in the 6-month fetus (Wirdnam and Milner, 1981).

Those with T1DM with pronounced β-cell autoimmunity born in Sweden or Berlin had a different seasonality of birth rate from that of others (Lewy *et al.*, 2008). The authors suggested that this finding was consistent with perinatal viral infections. Wintertime is the season of highest risk for developing T1DM in New Zealand (Willis *et al.*, 2002). That is when respiratory viral infections are most common, due largely to lower solar UVB doses (Cannell *et al.*, 2006).

An ecological study of T1DM incidence rates for 51 countries found a significant inverse correlation between solar UVB irradiance adjusted for both cloud cover and per capita health expenditure (Mohr *et al.*, 2008). A plot of T1DM incidence rate versus latitude produced a 'smiley' curve, with low rates near the equator and high rates at higher latitudes.

12.2.8 Rheumatoid arthritis

Evidence exists that RAis linked to low solar UVB/vitamin D. For example, a US study found highest rates of RA in the Northeast, where solar UVB doses in summer are lowest (Vieira *et al.*, 2010). A study in Italy inversely related RA disease activity and disability scores with 25(OH)D concentrations (Rossini, *et al.*, 2010).

12.2.9 Cardiovascular disease

For CVD, three lines of evidence suggest that solar UVB reduces risk. One is the seasonality of CVD mortality rates, highest in winter, lowest in summer (Scragg, 1981). The second comes from observational studies reporting reduced risk of CVD incidence or increased survival for those with higher serum 25(OH)D concentrations. The third is from direct correlations with some measure of UVB irradiance.

The period of peak CVD mortality rates in the northern hemisphere is December-March (Crawford *et al.*, 2003; Douglas *et al.*, 1991; Kendrovski, 2006; Tanaka *et al.*, 2000) and in the southern hemisphere, May-September (Barnett *et al.*, 2008; Weerasinghe *et al.*, 2002). These are the periods of lowest temperatures (Kendrovski, 2006) and lowest solar UVB doses and serum 25(OH)D concentrations (Hypponen and Power, 2007; Livesey *et al.*, 2007). Thus, separating the effects of temperature from those of UVB/vitamin D is difficult. An analogous situation exists for influenza: peak infection rates occur near December or January (Hope-Simpson, 1981), and studies have implicated both temperature/relative humidity (Shaman, 2011a,b) and solar UVB/vitamin D (Cannell *et al.*, 2006, 2008). Low temperature and relative humidity permit the influenza virus to survive longer outside a body, thereby increasing person-to-person transmission. Vitamin D reduced risk of type A influenza in one RCT (Urashima *et al.*, 2010). UV has one further effect on risk of CVD: lowering of blood pressure due to UVA-induced release of nitric oxide from cutaneous photolabile nitric oxide derivates (Oplander *et al.*, 2009).

Several studies have inversely correlated prediagnostic serum 25(OH)D concentrations with CVD incidence. A meta-analysis of 17 studies found an odds ratio of 0.67 (95% confidence interval, 0.55-0.81) for high versus low serum 25(OH)D for CVD incidence (Parker *et al.*, 2010).

An observational study from the Intermountain Healthcare system (based on 41,497 subjects with at least one vitamin D measurement from 2000 to 2009) examined the prevalence of CVD diagnoses for those older than 50 years for three serum 25(OH)D concentration ranges: less than 15 ng/ml, 16-30 ng/ml, and greater than 30 ng/ml. The study found statistically significant differences for all types of CVD: coronary heart disease, heart failure, atrial fibrillation, peripheral vascular disease, previous myocardial infarction, previous stroke, previous transient ischemic attack, and ventricular tachycardia (Anderson *et al.*, 2010).

Another observational study associated vitamin D supplementation with an odds ratio for death after diagnosis with CVD of 0.44 (95% confidence interval, 0.34-0.59) (Vacek *et al.*, 2011).

The mechanisms whereby vitamin D reduces the risk of CVD appear to include reduced risk of diabetes (Parker *et al.*, 2010), hypertension (Vaidya and Forman, 2012), infection and inflammation (Guillot *et al.*, 2010), and vascular calcification (Chang *et al.*, 2012; Rodriguez *et al.*, 2011; Zagura *et al.*, 2011).

Some studies have correlated higher altitude with lower risk of CVD. A Swiss study found that mortality rates for coronary heart disease decreased by 22% per 1000 m in altitude, whereas stroke mortality rates decreased by 12% per 1000 m in altitude (Faeh *et al.*, 2009). A Swedish study found reduced risk of venous thrombotic events for women by about 30% for several measures of UVB irradiance (Lindqvist *et al.*, 2009).

12.2.10 Anaphylaxis

The epidemiological evidence for a role of vitamin D comes largely from studies of the geographical variations in anaphylactic symptoms and seasonality of births among children with food allergies. The first epidemiological study was ecological, using regional differences in 2004 US EpiPen prescriptions (Camargo *et al.*, 2007). The highest rates were in the Northeast (8-12 prescriptions/1000 people), whereas the lowest rates were in the Southwest (2-3 prescriptions/1000 people). According to Figure 2 in that paper, the variation seems related to summertime solar UVB doses, which are highest in the Southwest and lowest in the Northeast (Fioletov *et al.*, 2010; Leffell and Brash, 1996). The distribution is highly asymmetric for two reasons: higher surface elevations in the western states and thinner stratospheric ozone layer in the West. The thin ozone layer is due to the prevailing westerly winds crossing the Rocky Mountains and pushing the tropopause higher. Solar UVB is the primary source of vitamin D for most Americans.

A similar study in Australia found a significant increase in EpiPen prescription rates going from 20°S to 45°S latitude (Mullins *et al.*, 2009). Because Australia has no mountain ranges, solar UVB doses decrease with increasing latitude. Other factors did not significantly affect the finding. A related study in Australia also found more use of hypoallergenic formula for infants in the southern and eastern regions (Mullins *et al.*, 2010).

A study of US emergency department hospital visits for acute allergic reactions found the highest rates in the Northeast, with a stronger association seen when the reactions were limited to those caused by food allergy (Rudders *et al.*, 2010).

Two studies of season of birth and food allergy in Boston children found higher rates for those born in fall or winter than in spring or summer. The first such study was conducted in the United States. It found a seasonal pattern, with lowest rates for emergency department patients younger than 5 years born in April to July and highest rates in December and January (Vassallo *et al.*, 2010). No seasonality was apparent for older children or adults. A second study involving 3,792 patients in Boston found the lowest rate for those younger than 18 years born in spring, with increasing relative ratio for summer, fall, and winter births (Vassallo and Camargo, 2010).

12.3 Causality

Much of the evidence that UV reduces the risk of disease comes from ecological and observational studies, with support from ecological, observational, and clinical studies on vitamin D. Many in the health system are used to having RCTs demonstrate efficacy and lack of harm for pharmaceutical drugs. Some have questioned this requirement for cancer prevention (Kristal, 2008). In fact, carrying out RCTs with UV irradiance would be difficult because solar UV is the primary source, and the lag time between exposure and disease outcome for chronic diseases is many years. Even for melanoma, which is linked to solar and artificial UV irradiance – more to sporadic than chronic irradiance (Gandini *et al.*, 2005; Chang *et al.*, 2009) – no RCTs have been conducted.

An alternative approach is to apply A.B. Hill's criteria for causality in a biological system (Biesalski *et al.*, 2011; Hill, 1965): strength of association, consistency, specificity (not applicable to vitamin D), temporality, biological gradient, plausibility (e.g. mechanisms), coherence, experiment (e.g. RCT), and analogy. Later work added accounting for confounding factors and avoidance of bias (Potischman and Weed, 1999). Several types of cancer (Grant, 2009a,b, 2012a,b), periodontal disease (Grant and Boucher, 2010), and MS (Hanwell and Banwell, 2011) generally satisfy these criteria.

The ecological approach is often considered the weakest type of epidemiological study. However, well-conducted ecological studies have often been the first type of study to identify and quantify a link between risk factors and disease outcome. Examples include dietary risks for cancer (Armstrong and Doll, 1975) and Alzheimer's disease (Grant, 1997), as well as UVB-vitamin D and cancer risk reduction (Garland and Garland, 1980). It is amusing that the finding of dietary fat as an important risk factor for many types of cancer (Armstrong and Doll, 1975) was opposed for many years until the realization that the effect of diet could occur early in life. When cohort studies included younger women, that meat and fat were risk factors for breast cancer (Cho *et al.*, 2006) became apparent.

12.4 Summary and conclusion

This chapter has summarized evidence that solar UVB, through production of vitamin D, reduces the risk of many types of chronic and infectious diseases. The research and journal literature on vitamin D is growing rapidly, so consider the discussion in this chapter an overview of the field as of early 2012. One can follow the literature as it develops by regularly searching the National Library of Medicine's PubMed database (http://www.pubmed.gov/).

Disclosure

I receive funding from the UV Foundation (McLean, VA, USA), Bio-Tech Pharmacal (Fayetteville, AR, USA), the Vitamin D Council (San Luis Obispo, CA, USA), and the Vitamin D Society (Canada).

References

Aloia, J.F. and Li-Ng, M., 2007. Re: epidemic influenza and vitamin D. Epidemiology and Infection 135, 1095-1096; author reply 1097-1098.

Anderson, J.L., May, H.T., Horne, B.D., Bair, T.L., Hall, N.L., Carlquist, J.F., Lappe, D.L. and Muhlestein, J.B., 2010. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. American Journal of Cardiology 106, 963-968.

Apperly, F.L., 1941. The relation of solar radiation to cancer mortality in North America. Cancer Research 1, 191-195.

Armitage, E.L., Aldhous, M.C., Anderson, N., Drummond, H.E., Riemersma, R.A., Ghosh, S. and Satsangi, J., 2004. Incidence of juvenile-onset Crohn's disease in Scotland: association with northern latitude and affluence. Gastroenterology 127, 1051-1057.

Armstrong, B. and Doll, R., 1975. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. International Journal of Cancer 15, 617-631.

Barnett, A.G., de Looper, M. and Fraser, J.F., 2008. The seasonality in heart failure deaths and total cardiovascular deaths. Australian and New Zealand Journal of Public Health 32, 408-413.

Berwick, M., Armstrong, B.K., Ben-Porat, L., Fine, J., Kricker, A., Eberle, C. and Barnhill, R., 2005. Sun exposure and mortality from melanoma. Journal of the National Cancer Institute 97, 195-199.

Biesalski, H.K., Aggett, P.J., Anton, R., Bernstein, P.S., Blumberg, J., Heaney, R.P., Henry, J., Nolan, J.M., Richardson, D.P., Van Ommen, B., Witkamp, R.F., Rijkers, G.T. and Zöllner, I., 2011. 26th Hohenheim Consensus Conference, September 11, 2010. Scientific substantiation of health claims: evidence-based nutrition. Nutrition 27, S1-20.

Boscoe, F.P. and Schymura, M.J., 2006. Solar ultraviolet-B exposure and cancer incidence and mortality in the United States, 1993-2002. BMC Cancer 6, 264.

Britten, R.H., 1932. The incidence of epidemic influenza, 1918-19. Public Health Reports 47, 303-339.

Camargo Jr., C.A., Clark, S., Kaplan, M.S., Lieberman, P. and Wood, R.A., 2007. Regional differences in EpiPen prescriptions in the United States: the potential role of vitamin D. Journal of Allergy and Clinical Immunology 120, 131-136.

Cannell, J.J., Vieth, R., Umhau, J.C., Holick, M.F., Grant, W.B., Madronich, S., Garland, C.F. and Giovannucci, E., 2006. Epidemic influenza and vitamin D. Epidemiology and Infection 134, 1129-1140.

Cannell, J.J., Zasloff, M., Garland, C.F., Scragg, R. and Giovannucci, E., 2008. On the epidemiology of influenza. Virology Journal 5, 29.

Chang, J.H., Ro, H., Kim, S., Lee, H.H., Chung, W. and Jung, J.Y., 2012. Study on the relationship between serum 25-hydroxyvitamin D levels and vascular calcification in hemodialysis patients with consideration of seasonal variation in vitamin D levels. Atherosclerosis 220, 563-568.

Chang, Y.M., Barrett, J.H., Bishop, D.T., Armstrong, B.K., Bataille, V., Bergman, W., Berwick, M., Bracci, P.M., Elwood, J.M., Ernstoff, M.S., Gallagher, R.P., Green, A.C., Gruis, N.A., Holly, E.A., Ingvar, C., Kanetsky, P.A., Karagas, M.R., Lee, T.K., Le Marchand, L., Mackie, R.M., Olsson, H., Osterlind, A., Rebbeck, T.R., Sasieni, P., Siskind, V., Swerdlow, A.J., Titus-Ernstoff, L., Zens, M.S. and Newton-Bishop, J.A., 2009. Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. International Journal of Epidemiology 38, 814-830.

Cheng, X.W., Lu, J., Wu, C.L., Yi, L.N., Xie, X., Shi, X.D., Fang, S.S., Zan, H., Kung, H.F. and He, M.L., 2011. Three fatal cases of pandemic 2009 influenza A virus infection in Shenzhen are associated with cytokine storm. Respiratory Physiology & Neurobiology 175, 185-187.

Cho, E., Spiegelman, D., Hunter, D.J., Chen, W.Y., Stampfer, M.J., Colditz, G.A. and Willett, W.C., 2003. Premenopausal fat intake and risk of breast cancer. Journal of the National Cancer Institute 95, 1079-1085.

Crawford, V.L., McCann, M. and Stout, R.W., 2003. Changes in seasonal deaths from myocardial infarction. QJM 96, 45-52.

Dal, H., Boldemann, C. and Lindelof, B., 2007. Does relative melanoma distribution by body site 1960-2004 reflect changes in intermittent exposure and intentional tanning in the Swedish population? European Journal of Dermatology 17, 428-434.

Dale, B.A. and Fredericks, L.P., 2005. Antimicrobial peptides in the oral environment: expression and function in health and disease. Current Issues in Molecular Biology 7, 119-133.

Danai, P.A., Sinha, S., Moss, M., Haber, M.J. and Martin, G.S., 2007. Seasonal variation in the epidemiology of sepsis. Critical Care Medicine 35, 410-415.

Devesa, S.S., Grauman, D.J., Blot, W.J., Pennello, G.A., Hoover, R.N. and Fraumeni Jr., J.F., 1999. Atlas of cancer mortality in the United States, 1950-1994. NIH Publication No. 99-4564.

Douglas, A.S., al-Sayer, H., Rawles, J.M. and Allan, T.M., 1991. Seasonality of disease in Kuwait. Lancet 337, 1393-1397.

Douglas, A.S., Brown, T. and Reid, D., 1996. Infectious mononucleosis and Hodgkin's disease – a similar seasonality. Leukemia and Lymphoma 23, 323-331.

East, B.R., 1939. Mean Annual Hours of Sunshine and the Incidence of Dental Caries. American Journal of Public Health and the Nations Health 29, 777-780.

Engel, P., Fagherazzi, G., Mesrine, S., Boutron-Ruault, M.C. and Clavel-Chapelon, F., 2011. Joint effects of dietary vitamin D and sun exposure on breast cancer risk: results from the French E3N cohort. Cancer Epidemiology, Biomarkers & Prevention 20, 187-198.

Faeh, D., Gutzwiller, F. and Bopp, M., 2009. Lower mortality from coronary heart disease and stroke at higher altitudes in Switzerland. Circulation 120, 495-501.

Field, S. and Newton-Bishop, J.A., 2011. Melanoma and vitamin D. Molecular Oncology 5, 197-214.

Fioletov, V.E., McArthur, L.J., Mathews, T.W. and Marrett, L., 2010. Estimated ultraviolet exposure levels for a sufficient vitamin D status in North America. Journal of Photochemistry and Photobiology. B: Biology 100, 57-66.

Flach, E.H., Rebecca, V.W., Herlyn, M., Smalley, K.S. and Anderson, A.R., 2011. Fibroblasts contribute to melanoma tumor growth and drug resistance. Molecular Pharmaceutics 8, 2039-2049.

Fleet, J.C., DeSmet, M., Johnson, R. and Li, Y., 2012. Vitamin D and cancer: a review of molecular mechanisms. Biochemical Journal 441, 61-76.

Gandini, S., Sera, F., Cattaruzza, M.S., Pasquini, P., Picconi, O., Boyle, P. and Melchi, C.F., 2005. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. European Journal of Cancer 41, 45-60.

Garland, C., Shekelle, R.B., Barrett-Connor, E., Criqui, M.H., Rossof, A.H. and Paul, O., 1985. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. Lancet 1, 307-309.

Garland, C.F., Comstock, G.W., Garland, F.C., Helsing, K.J., Shaw, E.K. and Gorham, E.D., 1989. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet 2, 1176-1178.

Garland, C.F. and Garland, F.C., 1980. Do sunlight and vitamin D reduce the likelihood of colon cancer? International Journal of Epidemiology 9, 227-231.

Garland, C.F., Gorham, E.D., Mohr, S.B. and Garland, F.C., 2009. Vitamin D for cancer prevention: global perspective. Annals of Epidemiology 19, 468-483.

Garland, F.C., Garland, C.F., Gorham, E.D. and Young, J.F., 1990. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. Preventive Medicine 19, 614-622.

Giovannucci, E., Liu, Y., Rimm, E.B., Hollis, B.W., Fuchs, C.S., Stampfer, M.J. and Willett, W.C., 2006. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. Journal of the National Cancer Institute 98, 451-459.

Gombart, A.F., 2009. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. Future Microbiology 4, 1151-1165.

Grant, W.B., 1997. Dietary links to Alzheimer's disease. Alzheimer's Disease Review 2, 42-55. Available at http://www.sunarc.org/JAD97.pdf.

Grant, W.B., 2002. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer 94, 1867-1875.

Grant, W.B., 2007a. An ecologic study of cancer mortality rates in Spain with respect to indices of solar UVB irradiance and smoking. International Journal of Cancer 120, 1123-1128.

Grant, W.B., 2007b. A meta-analysis of second cancers after a diagnosis of nonmelanoma skin cancer: additional evidence that solar ultraviolet-B irradiance reduces the risk of internal cancers. Journal of Steroid Biochemistry and Molecular Biology 103, 668-674.

Grant, W.B., 2008a. The effect of solar UVB doses and vitamin D production, skin cancer action spectra, and smoking in explaining links between skin cancers and solid tumours. European Journal of Cancer 44, 12-15.

Grant, W.B., 2008b. Hypothesis-Ultraviolet-B irradiance and vitamin D reduce the risk of viral infections and thus their sequelae, including autoimmune diseases and some cancers. Photochemistry and Photobiology 84, 356-365.

Grant, W.B., 2009a. How strong is the evidence that solar ultraviolet B and vitamin D reduce the risk of cancer?: An examination using Hill's criteria for causality. Dermato-Endocrinology 1, 17-24.

Grant, W.B., 2009b. Solar ultraviolet-B irradiance and vitamin D may reduce the risk of septicemia. Dermato-Endocrinology 1, 37-42.

Grant, W.B., 2010. An ecological study of cancer incidence and mortality rates in France with respect to latitude, an index for vitamin D production. Dermato-Endocrinology 2, 62-67.

Grant, W.B., 2011. A review of the role of solar ultraviolet-B irradiance and vitamin D in reducing risk of dental caries. Dermato-Endocrinology 3, 193-198.

Grant, W.B., 2012a. Ecological studies of the UVB-vitamin D-cancer hypothesis. Anticancer Research 32, 223-236.

Grant, W.B., 2012b. An ecological study of cancer mortality rates in California, 1950-64, with respect to solar UVB and smoking indices. Dermato-Endocrinology 4, 176-182.

Grant, W.B., 2012c. Role of solar UV irradiance and smoking in cancer as inferred from cancer incidence rates by occupation in Nordic countries. Dermato-Endocrinology 4, 203-211.

Grant, W.B. and Boucher, B.J., 2010. Are Hill's criteria for causality satisfied for vitamin D and periodontal disease? Dermato-Endocrinology 2, 30-36.

Grant, W.B. and Garland, C.F., 2006. The association of solar ultraviolet B (UVB) with reducing risk of cancer: multifactorial ecologic analysis of geographic variation in age-adjusted cancer mortality rates. Anticancer Research 26, 2687-2699.

Grant, W.B. and Giovannucci, E., 2009. The possible roles of solar ultraviolet-B radiation and vitamin D in reducing case-fatality rates from the 1918-1919 influenza pandemic in the United States. Dermato-Endocrinology 1, 215-219.

Grant, W.B. and Holick, M.F., 2005. Benefits and requirements of vitamin D for optimal health: a review. Alternative Medicine Review 10, 94-111.

Grant, W.B. and Mohr, S.B., 2009. Ecological studies of ultraviolet B, vitamin D and cancer since 2000. Annals of Epidemiology 19, 446-454.

Grant, W.B. and Peiris, A.N., 2010. Possible role of serum 25-hydroxyvitamin D in Black-White health disparities in the United States. Journal of the American Medical Directors Association 11, 617-628.

Grant, W.B. and Peiris, A.N., 2012. Differences in vitamin D status may account for unexplained disparities in cancer survival rates between African and White Americans. Dermato-Endocrinology 4, 85-94.

Guillot, X., Semerano, L., Saidenberg-Kermanac'h, N., Falgarone, G. and Boissier, M.C., 2010. Vitamin D and inflammation. Joint, Bone, Spine: Revue du Rhumatisme 77, 552-557.

Hanwell, H.E. and Banwell, B., 2011. Assessment of evidence for a protective role of vitamin D in multiple sclerosis. Biochimica et Biophysica Acta 1812, 202-212.

Hartge, P., Devesa, S.S., Grauman, D., Fears, T.R. and Fraumeni Jr., J.F., 1996. Non-Hodgkin's lymphoma and sunlight. Journal of the National Cancer Institute 88, 298-300.

Hill, A.B., 1965. The Environment and Disease: Association or Causation? Proceedings of the Royal Society of Medicine 58, 295-300.

Hjalgrim, H., Smedby, K.E., Rostgaard, K., Molin, D., Hamilton-Dutoit, S., Chang, E.T., Ralfkiaer, E., Sundstrom, C., Adami, H.O., Glimelius, B. and Melbye, M., 2007. Infectious mononucleosis, childhood social environment, and risk of Hodgkin lymphoma. Cancer Research 67, 2382-2388.

Hope-Simpson, R.E., 1981. The role of season in the epidemiology of influenza. Journal of Hygiene 86, 35-47.

Hypponen, E. and Power, C., 2007. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. American Journal of Clinical Nutrition 85, 860-868.

Jablonski, N.G. and Chaplin, G., 2000. The evolution of human skin coloration. Journal of Human Evolution 39, 57-106.

Jablonski, N.G. and Chaplin, G., 2010. Colloquium paper: human skin pigmentation as an adaptation to UV radiation. Proc Natl Acad Sci U S A 107 Suppl 2, 8962-8968.

Joseph, A.J., George, B., Pulimood, A.B., Seshadri, M.S. and Chacko, A., 2009. 25 (OH) vitamin D level in Crohn's disease: association with sun exposure & disease activity. Indian Journal of Medical Research 130, 133-137.

Kahn, H.S., Morgan, T.M., Case, L.D., Dabelea, D., Mayer-Davis, E.J., Lawrence, J.M., Marcovina, S.M. and Imperatore, G., 2009. Association of type 1 diabetes with month of birth among U.S. youth: The SEARCH for Diabetes in Youth Study. Diabetes Care 32, 2010-2015.

Kappelman, M.D., Rifas-Shiman, S.L., Kleinman, K., Ollendorf, D., Bousvaros, A., Grand, R.J. and Finkelstein, J.A., 2007. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clinical Gastroenterology and Hepatology 5, 1424-1429.

Kato, I., Tajima, K., Kuroishi, T. and Tominaga, S., 1985. Latitude and pancreatic cancer. Japanese Journal of Clinical Oncology 15, 403-413.

Kendrovski, V.T., 2006. The impact of ambient temperature on mortality among the urban population in Skopje, Macedonia during the period 1996-2000. BMC Public Health 6, 44.

Krishnan, A.V. and Feldman, D., 2011. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. Annual Review of Pharmacology and Toxicology 51, 311-336.

Kristal, A.R., 2008. Are clinical trials the "gold standard" for cancer prevention research? Cancer Epidemiology, Biomarkers & Prevention 17, 3289-3291.

Kurtzke, J.F., 1993. Epidemiologic evidence for multiple sclerosis as an infection. Clinical Microbiology Reviews 6, 382-427.

Lamprecht, S.A. and Lipkin, M., 2003. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nature Reviews: Cancer 3, 601-614.

Lappe, J.M., Travers-Gustafson, D., Davies, K.M., Recker, R.R. and Heaney, R.P., 2007. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. American Journal of Clinical Nutrition 85, 1586-1591.

Leffell, D.J. and Brash, D.E., 1996. Sunlight and skin cancer. Scientific American 275, 52-53, 56-59.

Lefkowitz, E.S. and Garland, C.F., 1994. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. International Journal of Epidemiology 23, 1133-1136.

Lewis, J.R., 1865. Exemptions from military service on account of loss of teeth. Dental Cosmos 7, 240-242.

Lewy, H., Hampe, C.S., Kordonouri, O., Haberland, H., Landin-Olsson, M., Torn, C. and Laron, Z., 2008. Seasonality of month of birth differs between type 1 diabetes patients with pronounced beta-cell autoimmunity and individuals with lesser or no beta-cell autoimmunity. Pediatric Diabetes 9, 46-52.

Lindqvist, P.G., Epstein, E. and Olsson, H., 2009. Does an active sun exposure habit lower the risk of venous thrombotic events? A D-lightful hypothesis. Journal of Thrombosis and Haemostasis 7, 605-610.

Liu, P.T., Stenger, S., Li, H., Wenzel, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., Kamen, D.L., Wagner, M., Bals, R., Steinmeyer, A., Zugel, U., Gallo, R.L., Eisenberg, D., Hewison, M., Hollis, B.W., Adams, J.S., Bloom, B.R. and Modlin, R.L., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311, 1770-1773.

Liu, P.T., Stenger, S., Tang, D.H. and Modlin, R.L., 2007. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. Journal of Immunology 179, 2060-2063.

Livesey, J., Elder, P., Ellis, M.J., McKenzie, R., Liley, B. and Florkowski, C., 2007. Seasonal variation in vitamin D levels in the Canterbury, New Zealand population in relation to available UV radiation. New Zealand Medical Journal 120, U2733.

Manaseki-Holland, S., Qader, G., Isaq Masher, M., Bruce, J., Zulf Mughal, M., Chandramohan, D. and Walraven, G., 2010. Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomised controlled trial. Tropical Medicine & International Health 15, 1148-1155.

McKinney, P.A., 2001. Seasonality of birth in patients with childhood Type I diabetes in 19 European regions. Diabetologia 44 Suppl 3, B67-74.

Mellanby, M. and Pattison, C.L., 1928. The Action of Vitamin D in Preventing the Spread and Promoting the Arrest of Caries in Children. British Medical Journal 2, 1079-1082.

Mohr, S.B., 2009. A brief history of vitamin D and cancer prevention. Annals of Epidemiology 19, 79-83.

Mohr, S.B., Garland, C.F., Gorham, E.D. and Garland, F.C., 2008. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. Diabetologia 51, 1391-1398.

Mookherjee, N., Rehaume, L.M. and Hancock, R.E., 2007. Cathelicidins and functional analogues as antisepsis molecules. Expert Opinion on Therapeutic Targets 11, 993-1004.

Mullins, R.J., Clark, S. and Camargo Jr., C.A., 2009. Regional variation in epinephrine autoinjector prescriptions in Australia: more evidence for the vitamin D-anaphylaxis hypothesis. Annals of Allergy, Asthma, and Immunology 103, 488-495.

Mullins, R.J., Clark, S. and Camargo Jr., C.A., 2010. Regional variation in infant hypoallergenic formula prescriptions in Australia. Pediatric Allergy and Immunology 21, e413-420.

Nerich, V., Monnet, E., Etienne, A., Louafi, S., Ramee, C., Rican, S., Weill, A., Vallier, N., Vanbockstael, V., Auleley, G.R., Allemand, H. and Carbonnel, F., 2006. Geographical variations of inflammatory bowel disease in France: a study based on national health insurance data. Inflammatory Bowel Diseases 12, 218-226.

Nerich, V., Monnet, E., Weill, A., Vallier, N., Vanbockstael, V., Auleley, G.R., Balaire, C., Dubost, P., Rican, S., Allemand, H. and Carbonnel, F., 2010. Fine-scale geographic variations of inflammatory bowel disease in France: correlation with socioeconomic and house equipment variables. Inflammatory Bowel Diseases 16, 813-821.

Oplander, C., Volkmar, C.M., Paunel-Gorgulu, A., van Faassen, E.E., Heiss, C., Kelm, M., Halmer, D., Murtz, M., Pallua, N. and Suschek, C.V., 2009. Whole body UVA irradiation lowers systemic blood pressure by release of nitric oxide from intracutaneous photolabile nitric oxide derivates. Circulation Research 105, 1031-1040.

Orton, S.M., Wald, L., Confavreux, C., Vukusic, S., Krohn, J.P., Ramagopalan, S.V., Herrera, B.M., Sadovnick, A.D. and Ebers, G.C., 2011. Association of UV radiation with multiple sclerosis prevalence and sex ratio in France. Neurology 76, 425-431.

Parker, J., Hashmi, O., Dutton, D., Mavrodaris, A., Stranges, S., Kandala, N.B., Clarke, A. and Franco, O.H., 2010. Levels of vitamin D and cardiometabolic disorders: systematic review and meta-analysis. Maturitas 65, 225-236.

Peller, S. and Stephenson, C.S., 1937. Skin irritation and cancer in the United states Navy. The American Journal of the Medical Sciences 194, 326-333.

Porojnicu, A., Robsahm, T.E., Berg, J.P. and Moan, J., 2007. Season of diagnosis is a predictor of cancer survival. Sun-induced vitamin D may be involved: a possible role of sun-induced Vitamin D. Journal of Steroid Biochemistry and Molecular Biology 103, 675-678.

Potischman, N. and Weed, D.L., 1999. Causal criteria in nutritional epidemiology. American Journal of Clinical Nutrition 69, 1309S-1314S.

Pukkala, E., Martinsen, J.I., Lynge, E., Gunnarsdottir, H.K., Sparen, P., Tryggvadottir, L., Weiderpass, E. and Kjaerheim, K., 2009. Occupation and cancer – follow-up of 15 million people in five Nordic countries. Acta Oncologica 48, 646-790.

Rajakumar, K., 2003. Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. Pediatrics 112, e132-135.

Rajakumar, K., Greenspan, S.L., Thomas, S.B. and Holick, M.F., 2007. SOLAR ultraviolet radiation and vitamin D: a historical perspective. American Journal of Public Health 97, 1746-1754.

Rajakumar, K. and Thomas, S.B., 2005. Reemerging nutritional rickets: a historical perspective. Archives of Pediatrics & Adolescent Medicine 159, 335-341.

Ramagopalan, S.V., Handel, A.E., Giovannoni, G., Rutherford Siegel, S., Ebers, G.C. and Chaplin, G., 2011. Relationship of UV exposure to prevalence of multiple sclerosis in England. Neurology 76, 1410-1414.

Rodriguez, M., Martinez-Moreno, J.M., Rodriguez-Ortiz, M.E., Munoz-Castaneda, J.R. and Almaden, Y., 2011. Vitamin D and vascular calcification in chronic kidney disease. Kidney and Blood Pressure Research 34, 261-268.

Rossini, M., Maddali Bongi, S., La Montagna, G., Minisola, G., Malavolta, N., Bernini, L., Cacace, E., Sinigaglia, L., Di Munno, O. and Adami, S., 2010. Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. Arthritis Research & Therapy 12, R216.

Rudders, S.A., Espinola, J.A. and Camargo Jr., C.A., 2010. North-south differences in US emergency department visits for acute allergic reactions. Annals of Allergy, Asthma, and Immunology 104, 413-416.

Sabetta, J.R., DePetrillo, P., Cipriani, R.J., Smardin, J., Burns, L.A. and Landry, M.L., 2010. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PloS One 5, e11088.

Schwartz, G.G. and Hulka, B.S., 1990. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Research 10, 1307-1311.

Scragg, R., 1981. Seasonality of cardiovascular disease mortality and the possible protective effect of ultra-violet radiation. International Journal of Epidemiology 10, 337-341.

Shaman, J., Goldstein, E. and Lipsitch, M., 2011a. Absolute humidity and pandemic versus epidemic influenza. American Journal of Epidemiology 173, 127-135.

Shaman, J., Jeon, C.Y., Giovannucci, E. and Lipsitch, M., 2011b. Shortcomings of vitamin D-based model simulations of seasonal influenza. PloS One 6, e20743.

Shaman, J., Pitzer, V.E, Viboud, C., Grenfel, B.T. and Lipsitch, M., 2010. Absolute humidity and the seasonal onset of influenza in the continental United States. PLoS Biology 8, e1000316.

Shivananda, S., Lennard-Jones, J., Logan, R., Fear, N., Price, A., Carpenter, L. and van Blankenstein, M., 1996. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). Gut 39, 690-697.

Sonnenberg, A., 2010. Similar geographic variations of mortality and hospitalization associated with IBD and Clostridium difficile colitis. Inflammatory Bowel Diseases 16, 487-493.

Tanaka, H., Shinjo, M., Tsukuma, H., Kawazuma, Y., Shimoji, S., Kinoshita, N. and Morita, T., 2000. Seasonal variation in mortality from ischemic heart disease and cerebrovascular disease in Okinawa and Osaka: the possible role of air temperature. Journal of Epidemiology 10, 392-398.

Tuohimaa, P., Pukkala, E., Scelo, G., Olsen, J.H., Brewster, D.H., Hemminki, K., Tracey, E., Weiderpass, E., Kliewer, E.V., Pompe-Kirn, V., McBride, M.L., Martos, C., Chia, K.S., Tonita, J.M., Jonasson, J.G., Boffetta, P. and Brennan, P., 2007. Does solar exposure, as indicated by the non-melanoma skin cancers, protect from solid cancers: vitamin D as a possible explanation. European Journal of Cancer 43, 1701-1712.

Urashima, M., Segawa, T., Okazaki, M., Kurihara, M., Wada, Y. and Ida, H., 2010. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. American Journal of Clinical Nutrition 91, 1255-1260.

Vacek, J.L., Vanga, S.R., Good, M., Lai, S.M., Lakkireddy, D. and Howard, P.A., 2012. Vitamin d deficiency and supplementation and relation to cardiovascular health. American Journal of Cardiology 109, 359-363.

Vaidya, A. and Forman, J.P., 2012. Vitamin D and Vascular Disease: The Current and Future Status of Vitamin D Therapy in Hypertension and Kidney Disease. Current Hypertension Reports 44, 111-119.

Van der Mei, I.A., Ponsonby, A.L., Dwyer, T., Blizzard, L., Simmons, R., Taylor, B.V., Butzkueven, H. and Kilpatrick, T., 2003. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ 327, 316.

Vassallo, M.F., Banerji, A., Rudders, S.A., Clark, S., Mullins, R.J. and Camargo Jr., C.A., 2010. Season of birth and food allergy in children. Annals of Allergy, Asthma, and Immunology 104, 307-313.

Vassallo, M.F. and Camargo Jr., C.A., 2010. Potential mechanisms for the hypothesized link between sunshine, vitamin D, and food allergy in children. Journal of Allergy and Clinical Immunology 126, 217-222.

Vieira, V.M., Hart, J.E., Webster, T.F., Weinberg, J., Puett, R., Laden, F., Costenbader, K.H. and Karlson, E.W., 2010. Association between residences in U.S. northern latitudes and rheumatoid arthritis: A spatial analysis of the Nurses' Health Study. Environmental Health Perspectives 118, 957-961.

Wallin, M.T., Page, W.F. and Kurtzke, J.F., 2004. Multiple sclerosis in US veterans of the Vietnam era and later military service: race, sex, and geography. Annals of Neurology 55, 65-71.

Walther, U., Kron, M., Sander, S., Sebastian, G., Sander, R., Peter, R.U., Meurer, M., Krahn, G. and Kaskel, P., 2004. Risk and protective factors for sporadic basal cell carcinoma: results of a two-centre case-control study in southern Germany. Clinical actinic elastosis may be a protective factor. British Journal of Dermatology 151, 170-178.

Weerasinghe, D.P., MacIntyre, C.R. and Rubin, G.L., 2002. Seasonality of coronary artery deaths in New South Wales, Australia. Heart 88, 30-34.

White, A.N., Ng, V., Spain, C.V., Johnson, C.C., Kinlin, L.M. and Fisman, D.N., 2009. Let the sun shine in: effects of ultraviolet radiation on invasive pneumococcal disease risk in Philadelphia, Pennsylvania. BMC Infectious Diseases 9, 196.

Willis, J.A., Scott, R.S., Darlow, B.A., Lewy, H., Ashkenazi, I. and Laron, Z., 2002. Seasonality of birth and onset of clinical disease in children and adolescents (0-19 years) with type 1 diabetes mellitus in Canterbury, New Zealand. Journal of Pediatric Endocrinology and Metabolism 15, 645-647.

Wirdnam, P.K. and Milner, R.D., 1981. Quantitation of the B and A cell fractions in human pancreas from early fetal life to puberty. Early Human Development 5, 299-309.

Zagura, M., Serg, M., Kampus, P., Zilmer, M., Eha, J., Unt, E., Lieberg, J., Cockcroft, J.R. and Kals, J., 2011. Aortic stiffness and vitamin D are independent markers of aortic calcification in patients with peripheral arterial disease and in healthy subjects. European Journal of Vascular and Endovascular Surgery 42, 689-695.

Key facts

- Multiple sclerosis, allergic asthma and type 1 diabetes are diseases driven by active immune processes, and downregulated by anti-inflammatory medications.
- There is a greater incidence of multiple sclerosis, allergic asthma and type 1 diabetes in populations living at higher latitudes (i.e. a positive latitude gradient).
- There are seasonal effects in expression of multiple sclerosis, allergic asthma and type 1 diabetes with highest incidence in winter.
- UV irradiation of skin stimulates suppression of immune responses both locally at the irradiated site and systemically with reduced responses to antigens administered to non-irradiated sites.
- All immune cells express a vitamin D receptor and can respond to vitamin D.

Summary points

- Ultraviolet radiation (UVR) exposure reduces immune responses to antigens, allergens and tumour antigens in humans.
- The immunosuppressive effects of UVR are used in phototherapy for treatment of skin inflammatory diseases such as psoriasis and atopic dermatitis.
- The extent of involvement of UVR-induced vitamin D in the immunosuppressive effects of UVR is unknown.
- Responses to vitamin D *in vitro* by immune cells are associated with reduced immunity.
- Inverse associations have been reported between incidence of chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes and serum vitamin D levels (i.e. increased disease if reduced vitamin D levels, reduced disease if higher vitamin D levels).
- Some but not all studies in mice made vitamin D deficient by dietary intervention have increased expression of symptoms associated with chronic immune diseases.
- Trials of vitamin D supplementation for chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes have been inconclusive.
- In murine studies, complementary as well as overlapping pathways are stimulated by vitamin D-dependent and vitamin D-independent pathways after UVR exposure.
- The importance in humans of UVR-induced pathways of immunosuppression that are not vitamin D-dependent remain unknown.
- Decisions on immunotherapeutic approaches for chronic immune diseases that target vitamin D-dependent or vitamin D-independent pathways must await the outcomes of several ongoing multi-centre large trials of vitamin D supplementation for disease control.

13. Immune modulation by UV: role of vitamin D

P.H. Hart and S. Gorman
Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, P.O. Box 855, West Perth 6872 Australia; prueh@ichr.uwa.edu.au

Abstract

A positive latitude gradient, namely a greater incidence of disease for individuals living at higher latitudes away from the equator, has been reported for chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes. Lower circulating levels of vitamin D have also been associated with increased prevalence of these diseases. As humans obtain more than 80% of their vitamin D by exposure of skin to the ultraviolet B (UVB) component of sunlight, it has been proposed that the two observations are linked. In this chapter, evidence for and evidence against the involvement of vitamin D in the immunoregulatory properties of UV radiation is presented. There are multiple photoreceptors in skin that absorb UVB photons, of which 7-dehydrocholesterol, the vitamin D precursor, is one. There may be many interacting and/or redundant pathways stimulated by ultraviolet radiation (UVR) exposure resulting in reduced immune responses. In mouse models both UVR and vitamin D are able to regulate immune responses but the potency of these effects *in vivo* is not fully understood. It is possible that levels of vitamin D are a biomarker of sun exposure and associations of disease incidence with circulating vitamin D levels have been incorrectly interpreted as dependent on the biological actions of vitamin D. Both vitamin D-dependent and vitamin D-independent pathways may account for the immunoregulatory effects of UVR. More research is needed. The most important test will be whether vitamin D supplementation can reduce the risk of diseases positively associated with latitude.

Keywords: multiple sclerosis, allergic asthma, type 1 diabetes, immunotherapy, latitude gradient

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_13, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
PGE_2	Prostaglandin E_2
RANKL	Receptor activator of NFkB ligand
UV	Ultraviolet
UVB	Ultraviolet B
UVR	Ultraviolet radiation (<400 nm)

13.1 Evidence for ultraviolet radiation-induced immunosuppression

The best evidence for UVR-induced immunosuppression comes from epidemiological observations of increased disease prevalence of chronic immune diseases with increasing latitude of residence, and by inference, less sun (UVR) exposure. Further, support for UVR-induced immunosuppression has come from seasonal changes in expression of immune diseases with greater symptoms of disease during winter when less sun exposure occurs. The expression of the debilitating autoimmune disease, multiple sclerosis, is an excellent example. The concordance rate for identical twins is 30% and suggests important environmental influences on disease development. Both seasonal variation in disease expression (Van der Mei *et al.*, 2011) and a positive latitude gradient have been reported for multiple sclerosis (Simpson *et al.*, 2011). Further, greater amounts of sun exposure may benefit patients of all ages with multiple sclerosis, although exposure during childhood may have particularly enhanced benefits on expression of disease.

Using latitude as a proxy measure for sun exposure, positive latitude gradients have also been reported for other chronic diseases characterised by overzealous immune reactivity. These include allergic asthma (Krstic, 2011) and type 1 diabetes. A positive latitude gradient may account for up to 40% of the variation in type 1 diabetes (Ponsonby *et al.*, 2005). Further, seasonal variation of diagnosis of type 1 diabetes with peaks in winter and troughs in summer has been reported (Moltchanova *et al.*, 2009).

In phototherapy, UVR is used to treat patients with inflammatory skin conditions, including psoriasis and atopic dermatitis. To complement the improvement of symptoms in summer, exposure to narrow band UVB (311-313 nm) can improve the symptoms of these conditions (Milliken *et al.*, 2012).

UVR-induced immunosuppression has also been implicated in the development of UVR-induced skin cancers. UVR-induced skin cancers are immunogenic and will be destroyed by a competent immune system. However, because UV is immunosuppressive, skin cancers continue to grow and develop. This has been highlighted by the increased skin cancer development in human patients on immunosuppressive therapy, for example in transplant patients. Supporting these findings is the evidence that exposure to UVR can interfere with outcomes of vaccination against poliovirus,

influenza virus, tuberculosis, hepatitis B virus, measles virus and rubella virus (reviewed by Norval and Woods, 2011). Seasonal effects of recall immune responses to vaccine antigens have been reported. After a holiday involving summer sun exposure, recall immune responses to standard multitest antigens were reduced when they were applied to both the sun-exposed and to the non-exposed skin (Norval and Woods, 2011).

Studies in mice have allowed a clearer dissection of the mechanisms of UVR-induced immunosuppression with measurement of cells, cytokines and other soluble mediators in UVR-exposed skin, the draining lymph nodes and in distant lymph nodes. Further, responses to UVR may depend on variables that relate to each individual (genetic makeup, skin colour, age, amount of skin exposed) as well as environmental factors other than season and latitude that may alter the intensity and spectral range of UV (time of day, pollution, cloud cover, ozone layer properties). The immunoregulatory properties of different wavelengths are a matter of much debate with UVA wavelengths (320-400 nm) reported to be both immunosuppressive and immunoprotective, and with different effects measured in response to different doses of UVR delivered. Upon measuring responses to nickel challenge in nickel-allergic individuals, UVB at 300 nm and UVA at 370 nm were immunosuppressive (Halliday *et al.*, 2012). UVB wavelengths (290-315 nm) however, remain the most potent and UVR-induced immunosuppression can be measured in the absence of erythema (redness of the skin and sunburn) (Hart *et al.*, 2011; Norval and Halliday, 2011; Ullrich and Byrne, 2012).

13.2 Ultraviolet radiation photoreceptors in skin

13.2.1 7-dehydrocholesterol

Upon absorption of UVB photons, 7-dehydrocholesterol of keratinocyte membranes is converted to pre-vitamin D3 which then thermally isomerises to vitamin D3 (cholecalciferol) (reviewed by Bouillon *et al.*, 2008). Much of the vitamin D3 is stepwise hydroxylated in the liver to 25(OH)D3, and in the kidney to $1,25(OH)_2D3$, the active form of vitamin D. In addition to this route, keratinocytes and immune cells in the skin form $1,25(OH)_2D3$ within 16 hours of UVR exposure. 25(OH)D3 is the storage form of vitamin D and representative of the vitamin D 'status' of an individual; it is the level typically used in studies investigating associations of serum vitamin D with disease outcomes. The amount of 25(OH)D3 produced upon UVB irradiation of skin varies for different individuals and can depend on your pre-exposure vitamin D levels; if you have a lower starting level, sunlight will stimulate greater increases in 25(OH)D3. Dark-skinned individuals are less efficient at making vitamin D. For most individuals, diet contributes in a minor way to vitamin D levels.

13.2.2 DNA, urocanic acid and membrane phospholipids

DNA changes characteristic of UVB exposure include the formation of cyclobutane pyrimidine dimers and pyrimidine(6-4)photoproducts. The UVA signature includes both cyclobutane

pyrimidine dimers as well as 8-oxo-7,8-dihydroguanine caused by UV-induced reactive oxygen species (Tewari *et al.*, 2012). The involvement of UV-induced DNA damage in the mechanisms of UVR-induced immunosuppression was supported by studies in which UV-induced suppression of contact hypersensitivity responses was not detected if the irradiated mice were treated with liposomes containing T4 endonuclease V, an excision repair enzyme. Further, the application of cytokines that can activate nucleotide excision repair can reduce UVR-induced immunosuppression (Schwarz and Schwarz, 2011).

Trans-urocanic acid in the stratum corneum isomerises to its *cis* isomer upon UVR exposure. *Cis*-urocanic acid initiates signalling pathways involving receptors for platelet activating factor, serotonin and histamine. Soluble mediators from keratinocytes, nerves, skin dendritic cells and mast cells have been implicated in the immunosuppressive events following *cis*-urocanic acid formation. The production of reactive oxygen species by *cis*-urocanic acid, resulting in oxidised DNA and protein has also been reported.

UVR may stimulate a stress response in the membranes of keratinocytes and other skin cells. This can be characterised by absorption of UV photons by tryptophan and the formation of ligands for the cytoplasmic aryl hydrocarbon receptor. Activation of this receptor increases the transcription of many genes, and increased production of immunoregulatory cytokines and mediators such as PGE_2 (Krutmann *et al.*, 2012).

13.2.3 Concluding statement

The extent of overlap and redundancy of the signalling pathways initiated by UV photoreceptors is not known. The purpose of this chapter is to discuss the relative importance of UVR-induced vitamin D in UVR-induced immunosuppression, particularly as it relates to the perceived control by UVR, as suggested by latitude gradients, of chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes.

13.3 Ulraviolet-induced vitamin D as a major contributor

13.3.1 Vitamin D associations and increased immune disease

For patients with multiple sclerosis, low serum 25(OH)D3 levels have been associated with increased risk of disease, progression of disease, and increased relapse rate (Holick, 2007). A recent meta-analysis of case-control studies concluded that polymorphisms in the vitamin D receptor gene are not associated with multiple sclerosis risk (Huang and Zie, 2012) although VDR variants have been reported to moderately modulate the risk of disease conferred by HLA-DRB1*15:01, a strong risk factor in females (Irizar *et al.*, 2011).

For paediatric and adult patients with allergic asthma, low serum 25(OH)D3 levels have correlated with increased allergen sensitivity (high IgE levels), bronchial hyperresponsiveness,

poor lung function and reduced responses to steroids (reviewed by Dimoloe *et al.*, 2010; Hollams *et al.*, 2011). In a community-based cohort study in Perth, Australia, the transition of children to an allergic asthma phenotype was studied; 25(OH)D3 levels at ages 6 and 14 were negatively associated with concurrent allergic phenotypes, particularly in boys (Hollams *et al.*, 2011). Vitamin D levels at age 6 were also significant predictors of subsequent atopy/asthma-associated phenotypes at age 14.

Similar negative associations of lower serum 25(OH)D3 levels and increased risk of diabetes in both children and adults have been published (Baeke *et al.*, 2010). For both asthma and type 1 diabetes, vitamin D receptor polymorphisms have been linked with risk of disease.

13.3.2 Use of mouse models of disease

The associations between vitamin D insufficiency and measures of prevalence and intensity of multiple sclerosis, allergic asthma and type 1 diabetes have not confirmed vitamin D insufficiency as the cause, rather than a consequence, of the disease. For this reason, regulation of murine models of these diseases has been studied. Mice genetically deficient in the vitamin D receptor or Cyp27b1, the enzyme responsible for hydroxylation of 25(OH)D3 to $1,25(OH)_2D3$, are available. However, these mice have serious developmental problems that lead to skeletal, reproductive and immune system dysfunction and abnormal skin physiology (Bouillon *et al.*, 2008). As they are unsuitable for studies of administration of UVR to skin, disease models have been studied in mice deficient or supplemented by dietary means with vitamin D.

The results from murine studies have not been definitive. UVR at a dose that stimulated a just perceptible erythema, delivered either before or after allergen sensitisation, reduced allergic airways disease in mice (Scott *et al.*, 2011). This supported the finding that there was increased expression of many aspects of allergic disease in mice made vitamin D deficient by dietary intervention (Gorman *et al.*, 2012). Thus, sufficient vitamin D and UVR-induced vitamin D may control disease expression. Similarly, vitamin D deficiency by dietary control early in life increased the incidence of diabetes in nonobese diabetic mice (Giulietti *et al.*, 2004) whilst chronic administration of $1,25(OH)_2D3$ induced suppressor cells and reduced disease outcomes (Mathieu *et al.*, 1994). With the murine model of multiple sclerosis based on autoimmune responses to myelin oligodendrocyte glycoprotein, chronic UVR, 25(OH)D3 and $1,25(OH)_2D3$ were all immunosuppressive, even if hypercalcaemia was induced by vitamin D (Becklund *et al.*, 2010). It is notable that in these studies, UVR was immunoregulatory of the multiple sclerosis model without induction of significant vitamin D above baseline levels.

13.3.3 Similar pathways of immunoregulation by UVR and vitamin D

If mechanisms of immunoregulation by UVR and vitamin D are similar, UVR may signal in large part by production of vitamin D. Alternatively, UVR may signal immunosuppressive pathways independently of vitamin D. Many of the cells and mediators involved in UVR-

induced immunosuppression will be discussed below and will be directly compared with those characterised in immunomodulation by $1,25(OH)_2D3$ binding to the vitamin D receptor.

13.3.4 Dendritic cells

Dendritic cells are very important sentinel cells that capture and process antigen and present it to T lymphocytes. Both UVR and topically applied vitamin D target dendritic cells of the skin. In local UVR-induced immunosuppression, it has been postulated that UVR-damaged dendritic cells with oxidative damage to their DNA, membrane lipids and protein, 'limp' to the draining lymph nodes and present antigen in an altered way such that T regulatory cells are induced (Schwarz and Schwarz, 2011). Similarly upon application of $1,25(OH)_2D3$ to skin, the priming ability of draining lymph node dendritic cells is subverted (Gorman *et al.*, 2010a). In this latter study, dendritic cells from the draining lymph nodes of $1,25(OH)_2D3$-treated mice induced significantly smaller ear-swelling responses in a contact hypersensitivity reaction. Further, T regulatory CD4+CD25+ cells isolated from the auricular lymph nodes of mice that received ear injections of dendritic cells from donor mice topically treated with $1,25(OH)_2D3$ more potently suppressed effector cell proliferation. Finally, dendritic cells from the draining lymph nodes of $1,25(OH)_2D3$-treated mice expressed increased levels of indoleamine 2,3-dioxygenase mRNA, and suggested that pathway by which $1,25(OH)_2D3$ may regulate dendritic cell function (Gorman *et al.*, 2010a).

13.3.5 Increased expression of the receptor activator of NFkB ligand

RANKL expression in keratinocytes increases upon UVR exposure or after topical application of vitamin D (Ghoreishi *et al.*, 2009) and has been implicated in expansion of antigen specific T regulatory cells. In response to a UVR-induced, vitamin D-dependent process, it is proposed that dendritic cells migrate to the draining nodes and augment the number and suppressive activity of lymph node resident regulatory T lymphocytes. Ghoreishi and colleagues were unable to detect this effect of UVR in mice without a functional vitamin D receptor (Ghoreishi *et al.*, 2009) which strongly implicated a vitamin D-dependent pathway. In another model, UVR-induced PGE_2 also stimulated epidermal RANKL expression and in turn, increased numbers of both dendritic cells and T regulatory cells in the draining nodes (Soontrapa *et al.*, 2011). It is plausible that UVR-induced vitamin D and PGE_2 have synergistic functions.

13.3.6 Induction and activation of T regulatory cells

It is generally proposed that UVR-damaged dendritic cells are tolerogenic and induce antigen-specific T regulatory cells (Schwarz and Schwarz, 2011). However, not all UVR-induced CD4+ T regulatory cells are antigen specific. CD4+CD25+ cell from the nodes of UV-irradiated skin can supress immune responses to new antigens both *in vitro* and *in vivo* (Gorman *et al.*, 2007a). As topically applied $1,25(OH)_2D3$ led to the same outcome (Gorman *et al.*, 2007b), this activation of naturally occurring T regulatory cells in the draining lymph nodes of UVR-treated skin is probably due to UVR-induced vitamin D3. It is of note that dendritic cells may not always be the

conduit by which 1,25$(OH)_2$D3 increases T regulatory cell activity. It has recently been reported that 1,25$(OH)_2$D3 can directly promote *FOXP3* expression, an important functional aspect of T regulatory cells, by binding to vitamin D response elements in its conserved noncoding sequence region (Kang *et al.*, 2012).

13.3.7 Activation of mast cells

Dermal mast cells are important to the mechanisms by which UVR causes a systemic immunosuppression. Murine strains that are very sensitive to the immunoregulatory properties of UVR (e.g. C57BL/6) have a high mast cell prevalence in skin whilst those that require larger doses of UVR to be immunosuppressive (e.g. BALB/c) have a relatively low mast cell prevalence in their skin (Hart *et al.*, 1998). Further, mast cell-deficient mice are unable to be immunosuppressed by UVR unless mast cell progenitors are transplanted into skin that is subsequently UV irradiated (Hart *et al.*, 1998). Most importantly, if the mast cell progenitors are derived from mice genetically deficient in the vitamin D receptor, they are unable to respond to UVR (Biggs *et al.*, 2010). The ability of mast cells to produce immunoregulatory IL-10 in response to 1,25$(OH)_2$D3 was also demonstrated (Biggs *et al.*, 2010). Thus, UVR-induced mast cell activation may depend on UVR-induced vitamin D production.

13.3.8 Local skin effects and responses to vaccine antigens

The efficacy of topical vaccination may be reduced if applied to UV-irradiated skin. Of note, responses to immunisation were more reduced if the antigen was applied to irradiated skin 24 hours after UVR exposure, and was associated with UVR-induced activation of Cyp27b1, the enzyme responsible for hydroxylation of 25(OH)D3 to 1,25$(OH)_2$D3 (Enioutina *et al.*, 2002). Similarly, delivery of 1,25$(OH)_2$D3 at the time of allergen sensitisation can reduce later responses to allergen challenge (Taher *et al.*, 2008).

13.3.9 Summary

Negative associations of serum 25(OH)D3 levels with disease prevalence, and intensity of disease, suggest UVR-induced vitamin D may be responsible in large part for the latitude gradients reported for chronic immune diseases such as multiple sclerosis, asthma and type 1 diabetes. Many common pathways exist by which UVR and vitamin D can regulate immune processes in human and murine skin. Thus, one could propose that vitamin D may provide an inexpensive mediator to control chronic immune diseases. However, as will be discussed in the next section, supplementation of patients with vitamin D has not yet endorsed this conclusion.

13.4 Ultraviolet-induced vitamin D as a minor contributor

13.4.1 Outcome of vitamin D supplemention

In discussion of the outcome of any trials of vitamin D supplementation, the dose of vitamin D used, and whether supplemented with calcium, must be considered. In November 2010, the Institute of Medicine (USA) recommended a daily vitamin D allowance of 600 IU/day until 71 years of age, and 800 IU/day when older (Ross *et al.*, 2011). However, members of the Endocrine Societies have recommended vitamin D supplements of up to 4,000 IU/day (Holick *et al.*, 2011). It has been calculated that assuming a linear relationship at least up to 2,000 IU/day, 600 IU/day and 2,000 IU/day should increase 25(OH) vitamin D3 levels by 10 nmol/l (4 ng/ml) and 32 nmol/l (12.8 ng/ml), respectively (Agency for Healthcare Research and Quality, 2007).

For treatment of patients with multiple sclerosis, there are many ongoing trials with vitamin D. The low incidence rate of the disease necessitates multi-centre participation and many years to complete. In one trial, some benefit was reported when vitamin D was given as an add-on therapy with interferon β-1b. However, generally the benefits of vitamin D therapy for multiple sclerosis have been modest (Smolders *et al.*, 2011). For asthma, some encouraging but small supplementation studies have been reported for both newly diagnosed patients (Majak *et al.*, 2011) and for patients already on steroids and other medications (Urashima *et al.*, 2010; Xystrakis *et al.*, 2006). Several investigators have supplemented pregnant women with vitamin D; however, reduced allergic asthma in the children has not been consistently measured (Wjst, 2012). For control of type 1 diabetes in children, variable responses have been measured. Similar limited success has been measured in adult patients with type 2 obesity-related diabetes. In post hoc analyses from eight trials among participants with normal glucose tolerance at baseline and in three small underpowered trials of patients with established type 2 diabetes, there was no effect of vitamin D supplementation on glycaemic outcomes (Mitri *et al.*, 2011).

13.4.2 Further discordance between ultraviolet radiation and vitamin D

A recent study in patients with skin disease examined the effects of narrowband UVB light (311 nm) on human serum 25(OH)D3 and systemic immune function (Milliken *et al.*, 2012). In the 24 patients studied, UV treatment increased T regulatory cell numbers and was associated with reduced proliferative and IL-10 responses to anti-CD3/CD28. UV also increased serum vitamin D levels but the increases were independent of the immune changes. The authors concluded that UVB light and serum vitamin D levels may affect particular immune functions independently (Milliken *et al.*, 2012).

In the murine models of multiple sclerosis, both 25(OH)D3 and $1,25(OH)_2D3$ regulated disease expression (Becklund *et al.*, 2010). However, the hypercalcaemia induced would be unacceptable in human trials. Further there was no suppression of the disease outcomes if the hypercalcaemia was controlled (Cantorna *et al.*, 1999). In contrast, UVR was immunosuppressive without altering serum calcium levels. If vitamin D deficiency is part of the pathogenesis of multiple sclerosis, the

disease model should be worse in vitamin D-deficient mice. Contrary results, namely less disease in vitamin D-deficient animals, have been published (De Luca and Plum, 2011; Fernandes de Abreu *et al.*, 2010).

In models of asthma, a conclusion that UVR-induced vitamin D is responsible for UVR-induced regulation of disease outcomes may be premature. Mice without functional vitamin D receptors, express no overt disease under conditions in which wild type mice develop experimental allergic airways disease (Wittke *et al.*, 2007). Further, vitamin D may have a greater effect on lung development than on the immunological processes associated with disease expression upon allergen exposure (Zosky *et al.*, 2011).

Topically applied $1,25(OH)_2D3$ can decrease UVR-induced DNA damage, and repair the cyclobutane pyrimidine dimers. It is a little controversial however, as to whether this repair of DNA reverses UVR-induced systemic immunosuppression. A reduction of the extent of suppression of contact hypersensitivity responses by UVR by topically applied $1,25(OH)_2D3$ was reported by one group (Mason *et al.*, 2010) whereas in our laboratory, a consistent augmentation of the extent of suppression is measured if the shaved dorsal skin of mice is given both UVR and $1,25(OH)_2D3$ (Gorman *et al.*, 2010b). It is possible that $1,25(OH)_2D3$ is reversing the effects of UVR by neutralising some of the effects of UV-induced nitric oxide (Mason *et al.*, 2010). In human skin topical $1,25(OH)_2D3$ does not reverse UVR-induced immunosuppression (Damian *et al.*, 2010). Although we do not fully understand the biochemical processes, all the results (antagonism or synergy) suggest that the pathway by which UVR suppresses contact hypersensitivity responses is not largely dependent on formation of $1,25(OH)_2D3$.

There is a further newly recognised pathway by which UVR may be immunoregulatory and may be important in UVR control of multiple sclerosis, allergic asthma and type 1 diabetes. In these studies, erythemal UVR stimulated the production of poorly immunogenic dendritic cells from bone marrow (Ng *et al.*, 2010). This may reflect a more sustained response to UVR compared with other immune alterations as the bone marrow is the site of development of many haemopoietic lineages for subsequent migration out to peripheral organs and for the rejuvenation and replacement of immune cells during inflammation. This depends on the inflammatory nature of erythemal UVR on skin and would be independent of the actions of UVR-induced vitamin D. This process is dependent on the production of prostanoids, particularly PGE_2 (Ng *et al.*, 2010; Scott *et al.*, 2012).

Further research is required but not all UV wavelengths that can signal UVR-induced immunosuppression can induce vitamin D production in skin. The universally recognised action spectrum for the UV-induced conversion of 7-dehydrocholesterol to pre-vitamin D3 in human skin indicates a maximum at about 297 nm with essentially no production above 315 nm. Although the validity of this action spectrum is being questioned (Norval *et al.*, 2010), UVR can be immunosuppressive at larger wavelengths that would not involve vitamin D production (Halliday *et al.*, 2012).

13.5 Conclusion

A strong case has been outlined above suggesting that vitamin D may be responsible for many of the immunosuppressive properties of UVR, particularly in response to UVB wavelengths. Many similar and over-lapping pathways are stimulated in UVB-treated skin and skin topically applied with $1,25(OH)_2D3$. Many cells implicated in UVR-induced immunosuppression have similar properties in response to UVR and $1,25(OH)_2D3$. Further, there are examples where UVR was not effective in mice engrafted with cells not expressing the vitamin D receptor. On the other hand, there are other immune pathways that are proposed important in UVR-induced immunosuppression that are not stimulated by $1,25(OH)_2D3$. Although there is no evidence that similar pathways do not occur in human skin, it is of note that many of the studies of the functional properties of UVR and $1,25(OH)_2D3$ have been completed in mice. It is also possible that some of the benefits attributed to UVR exposure and/or higher vitamin D levels in patients with multiple sclerosis, allergic asthma and type 1 diabetes may be due to the exercise that they receive outside and vitamin D levels are a proxy measure of this exercise in the sunshine (Maxwell and Wood, 2011).

It is reasonable to conclude that both complementary as well as over-lapping pathways are stimulated by vitamin D-dependent and vitamin D-independent pathways after UVR exposure. It is a matter of debate and ongoing research as to where the balance lies. It is notable that both sun exposure and vitamin D are independent risk factors for demyelination of the central nervous system (Lucas *et al.*, 2011). Perhaps more importantly, the balance relates to the identification of the pathways that are responsible for UVR-regulation, and by proxy the latitude gradients, for suppressing the incidence and progression of chronic immune diseases such as multiple sclerosis, allergic asthma and type 1 diabetes. It is also important in formulating advice that can be given by physicians to their patients with these diseases. Should they recommend vitamin D supplementation or extended periods of moderate sun exposure?

To answer this question, large multi-centre randomised controlled trials are required of patients given vitamin D3. The dose of supplemental vitamin D3 will be important, as well as the stage and intensity of the disease during which the vitamin D3 supplements are given. Trials to date may not have been powered sufficiently for measures of change in these complex immune diseases. The next few years will be very important for determining an answer to the title of this chapter, namely the role of vitamin D in immune modulation by UV. If trials suggest that vitamin D can efficiently regulate chronic immune diseases, immune modulation by UVR indicated by latitude gradients and disease relief in summer may reflect, at least in part, UVR control by a vitamin D-dependent pathway.

Acknowledgements

Our research has been supported by the National Health and Medical Research Council of Australia, the Cancer Council Western Australia and the Asthma Foundation of Western

Australia. PHH is supported by a Principal Research Fellowship from the National Health and Medical Research Council of Australia. SG is supported by a Fellowship from the Brightspark Foundation (Australia).

References

Agency for Healthcare Research and Quality, 2007. Effectiveness and Safety of vitamin D in relation to bone health. Rockville, Maryland: Agency for Healthcare Research and Quality; Report No. 07-E013.

Baeke, F., Takiishi, T., Korf, H., Gysemans, C. and Mathieu, C., 2010. Vitamin D: modulator of the immune system. Current Opinion in Pharmacology 10, 482-496.

Becklund, B.R., Severson, K.S., Vang, S.V. and DeLuca, H.F., 2010. UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. Proceedings of the National Academy of Sciences of the USA 107, 6418-6423.

Biggs, L., Yu, C., Fedoric, B., Lopez, A.F., Galli, S.J. and Grimbaldeston, M.A., 2010. Evidence that vitamin D3 promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. Journal of Experimental Medicine 207, 455-463.

Bouillon, R., Carmeliet, G., Verlinden, L., van Etten, E., Verstuyf, A., Luderer, H.F., Lieben, L., Mathieu, C. and Demay, M., 2008. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocrine Reviews 29, 726-776.

Cantorna, M.T., Humpal-Winter, J. and DeLuca, H.F., 1999. Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. Journal of Nutrition 129, 1966-1971.

Damian, D.L., Kim, Y.J., Dixon, K.M., Halliday, G.M., Javeri, A. and Mason, R.S., 2010. Topical calcitriol protects from UV-induced genetic damage but suppresses cutaneous immunity in humans. Experimental Dermatology 19, e23-30.

DeLuca, H.F. and Plum, L.A., 2011. Vitamin D deficiency diminishes the severity and delays onset of experimental autoimmune encephalomyelitis. Archives of Biochemistry and Biophysics 513, 140-143.

Dimeloe, S., Nanzer, A., Ryanna, K. and Hawrylowicz, C., 2010. Regulatory T cells, inflammation and the allergic response – the role of glucocorticoids and vitamin D. Journal of Steroid Biochemistry and Molecular Biology 120, 86-95.

Enioutina, E.Y., Visic, D.M. and Daynes, R.A., 2002. The induction of systemic and mucosal immunity to protein vaccines delivered through skin sites exposed to UVB. Vaccine 20, 2116-2130.

Fernandes de Abreu, D.A., Ibrahim, E.C., Boucraut, J., Khrestchatisky, M. and Feron, F., 2010. Severity of experimental autoimmune encephalomyelitis is unexpectedly reduced in mice born to vitamin D-deficient mothers. Journal of Steroid Biochemistry and Molecular Biology 121, 250-253.

Ghoreishi, M., Liu, M.C. and Camargo, C.A., 2009. Expansion of antigen-specific regulatory T cells with the topical vitamin D analog calcipotriol. Journal of Immunology 182, 6071-6078.

Giulietti, A., Gysemans, C., Stoffels, K., van Etten, E., DeCallonne, B., Overbergh, L., Bouillon, R. and Mathieu, C., 2004. Vitamin D deficiency in early life accelerates type 1 diabetes in non-obese diabetic mice. Diabetologia 47, 451-462.

Gorman, S., Judge, M.A. and Hart, P.H., 2010a. Topical 1,25-dihydroxyvitamin D3 subverts the priming ability of draining lymph node dendritic cells. Immunology 131, 415-425.

Gorman, S., Judge, M.A. and Hart, P.H., 2010b. Immune-modifying properties of topical vitamin D: focus on dendritic cells and T cells. Journal of Steroid Biochemistry and Molecular Biology 121, 247-249.

Gorman, S., Kuritzky, L.A., Judge, M.A., Dixon, K.M., McGlade, J.P., Mason, R.S., Finlay-Jones, J.J. and Hart, P.H., 2007b. Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. Journal of Immunology 179, 6273-6283.

Gorman, S., Tan, D.H.W., Lambert, M.J.M., Scott, N.M., Judge, M.A. and Hart, P.H., 2012. Vitamin D3 deficiency enhances allergen-induced lymphocyte responses in a mouse model of allergic airway disease. Pediatric Allergy and Immunology 23, 83-87.

Gorman, S., Tan, J., Yerkovich, S.T., Finlay-Jones, J.J. and Hart, P.H., 2007a. CD4+ T cells in lymph nodes of UVB-irradiated mice suppress immune responses to new antigens both *in vitro* and *in vivo*. Journal of Investigative Dermatology 127, 915-924.

Halliday, G.M., Damian, D.L., Rana, S. and Byrne, S.N., 2012. The suppressive effects of ultraviolet radiation on immunity in the skin and internal organs: Implications for autoimmunity. Journal of Dermatological Science 66, 176-182.

Hart, P.H., Gorman, S. and Finlay-Jones, J.J., 2011. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? Nature Reviews Immunology 11, 584-596.

Hart, P.H., Grimbaldeston, M.A., Swift, G.J., Jaksic, A., Noonan, F.P. and Finlay-Jones, J.J., 1998. Dermal mast cells determine susceptibility to ultraviolet B-induced systemic suppression of contact hypersensitivity responses in mice. Journal of Experimental Medicine 187, 2045-2053.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M., 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. Journal of Clinical and Endocrine Metabolism 96, 1911-1930.

Hollams, E.M., Hart, P.H., Holt, B.J., Serralha, M., Parsons, F., de Klerk, N.H., Zhang, G., Sly, P.D. and Holt, P.G., 2011. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. European Respiratory Journal 38, 1320-1327.

Huang, J. and Xie, Z-F., 2012. Polymorphisms in the vitamin D receptor gene and multiple sclerosis risk: A meta-analysis of case-control studies. Journal of Neurological Science 313, 79-85.

Irizar, H., Munoz-Culla, M., Zuriarran, O., Goyenechea, E., Castillo-Trivino, T., Prada, A., Saenz-Cuesta, M.A., De Juan, D., Lopez de Munain, A., Olascoaga, J. and Otaegui, D., 2011. HLA-DRB1*15:01 and multiple sclerosis: a female association? Multiple Sclerosis 18, 569-577.

Kang, S.W., Kim, S.H., Lee, N., Lee, W-W., Hwang, K-A., Shin, M.S., Lee, S-H., Kim, W-U. and Kang, I., 2012. 1,25-dihydroxyvitamin D3 promotes *FOXP3* expression via binding to vitamin D response elements in its conserved noncoding sequence region. Journal of Immunology 188, 5276-5282.

Krstic, G., 2011. Asthma prevalence associated with geographical latitude and regional insolation in the United States of America and Australia. PLoS ONE 6:e18492.

Krutmann, J., Morita, A. and Chung, J.H., 2012. Sun exposure: what molecular photodermatology tells us about its good and bad sides. Journal of Investigative Dermatology 132, 976-84.

Lucas, R.M., Ponsonby, A.-L., Dear, K., Valery, P.C., Pender, M.P., Taylor, B.V., Kilpatrick, T.J., Dwyer, T., Coulthard, A., Chapman, C., van der Mei, I., Williams, D. and McMichael, A.J., 2011. Sun exposure and vitamin D are independent risk factors for CNS demyelination. Neurology 76, 540-548.

Majak, P., Olszowiec-Clebna, M., Smejda, K. and Stelmach, I., 2011. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. Journal of Allergy and Clinical Immunology 127, 1294-1296.

Mason, R.S., Sequeira, V.B., Dixon, K.M., Gordon-Thomson, C., Pobre, K., Dilley, A., Mizwicki, M.T., Norman, A.W., Feldman, D., Halliday, G.M. and Reeve, V.E., 2010. Photoprotection by 1alpha,25-dihydroxyvitamin D and analogs: further studies on mechanisms and implications for UV-damage. Journal of Steroid Biochemistry and Molecular Biology 121, 164-168.

Mathieu, C., Waer, M., Laureys, J., Rutgeerts, O. and Bouillon, R., 1994. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 37, 552-558.

Maxwell, C.S. and Wood, R.J., 2011. Update on vitamin D and type 2 diabetes. Nutrition Reviews 69, 291-295.

Milliken, S.V.I., Wassall, H., Lewis, B.J., Logie, J., Barker, R.N., Macdonald, H., Vickers, M.A. and Ormerod, A.D., 2012. Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function. Journal of Allergy and Clinical Immunology 129, 1554-1561.

Mitri, J., Muraru, M.D. and Pittas, A.G., 2011. Vitamin D and type 2 diabetes: a systematic review. European Journal of Clinical Nutrition 65, 1005-1015.

Moltchanova, E.V., Schreier, N., Lammi, N. and Karvonen, M., 2009. Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwide. Diabetes Medicine 26, 673-678.

Ng, R.L.X., Bisley, J.L., Gorman, S., Norval, M. and Hart, P.H., 2010. UV-irradiation of mice reduces the competency of bone marrow-derived CD11c+ cells via an indomethacin-inhibitable pathway. Journal of Immunology 185, 7207-7215.

Norval, M. and Halliday, G.M., 2011. The consequences of UV-induced immunosuppression for human health. Photochemistry and Photobiology 87, 965-977.

Norval, M. and Woods, G.M., 2011. UV-induced immunosuppression and the efficacy of vaccination. Photochemistry and Photobiological Science 10, 1267-1274.

Norval, M., Bjorn, L.O. and de Gruijl, F.R., 2010. Is the action spectrum for the UV-induced production of previtamin D3 in human skin correct? Photochemical and Photobiological Sciences 9, 11-17.

Ponsonby, A.L., Lucas, R.M. and van der Mei, I.A.F., 2005. UVR, vitamin D and three autoimmune diseases – Multiple sclerosis, type I diabetes, rheumatoid arthritis. Photochemistry and Photobiology 81, 1267-1275.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. Journal of Clinical and Endocrine Metabolism. 96, 53-58.

Schwarz, T. and Schwarz, A., 2011. Molecular mechanisms of ultraviolet radiation-induced immunosuppression. European Journal of Cell Biology 90, 560-564.

Scott, N.M., Lambert, M.J.M., Gorman, S., McGlade, J.P. and Hart, P.H., 2011. Differences in control by UV radiation of inflammatory airways disease in naïve and allergen pre-sensitised mice. Photochemical and Photobiological Sciences 10, 894-901.

Scott, N.M., Ng, R.L.X., Strickland, D.H., Bisley, J.L., Bazely, S.A., Gorman, S., Norval, M. and Hart, P.H., 2012. Towards homeostasis: regulatory dendritic cells from the bone marrow of mice with inflammation of the airways and peritoneal cavity. American Journal of Pathology 181(2), 535-547.

Simpson, S., Blizzard, L., Otahal, P., Van der Mei, I. and Taylor, B., 2011. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. Journal of Neurology Neurosurgery and Psychiatry 82, 1132-1141.

Smolders, J., Hupperts, R., Barkhof, F., Grimaldi, L.M., Holmoy, T., Killestein, J., Rieckmann, P., Schluep, M., Vieth, R., Hostalek, U., Ghazi-Visser, L. and Beelke, M.; SOLAR study group., 2011. Efficacy of vitamin D3 as add-on therapy in patients with relapsing-remitting multiple sclerosis receiving subcutaneous interferon beta-1a: a Phase II, multicenter, double-blind, randomized, placebo-controlled trial. Journal of Neurological Science 311, 44-49.

Soontrapa, K., Honda, T., Sakata, D., Yao, C., Hirata, T., Hori, S., Matsuoka, T., Kita, Y., Shimizu, T., Kabashima, K. and Narumiya, S., 2011. Prostaglandin E2-prostaglandin E receptor subtype 4 (EP4) signaling mediates UV irradiation-induced systemic immunosuppression. Proceedings of the National Academy of Sciences USA 108, 6668-6673.

Taher, Y.A., van Esch, B.C., Hofman, G.A., Henricks, P.A. and van Oosterhout, A.J., 2008. 1alpha,25-dihydroxyvitamin D3 potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF-beta. Journal of Immunology 180, 5211-5221.

Tewari, A., Sarkay, R.P. and Young, A.R., 2012. UVAI induces cyclobutane pyrimidine dimers but not 6-4 photoproducts in human skin *in vivo*. Journal of Investigative Dermatology 132, 394-400.

Ullrich, S.E. and Byrne, S.N., 2012. The immunologic revolution: photoimmunology. Journal of Investigative Dermatology 132, 896-905.

Urashima, M., Segawa, T., Okazaki, M., Kurihara, M., Wada, Y. and Ida, H., 2010. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. American Journal of Clinical Nutrition 91, 1255-1260.

Van der Mei, I.A., Simpson, S., Stankovich, J. and Taylor, B.V., 2011. Individual and joint action of environmental factors and risk of MS. Neurology Clinics 29, 233-255.

Wittke, A., Chang, A., Froicu, M., Harandi, O.F., Weaver, V., August, A., Paulson, R.F. and Cantorna, M.T., 2007. Vitamin D receptor expression by the lung micro-environment is required for maximal induction of lung inflammation. Archives in Biochemistry and Biophysics 460, 360-316.

Wjst, M., 2012. Is vitamin D supplementation responsible for the allergy epidemic? Current Opinions in Allergy and Clinical Immunology 12, 257-262.

Xystrakis, E., Kusumakar, S., Boswell, S., Peek, E., Urry, Z., Richards, D.F., Adikibi, T., Pridgeon, C., Dallman, M., Loke, T.K., Robinson, D.S., Barrat, F.J., O'Garra, A., Lavender, P., Lee, T.H., Corrigan, C. and Hawrylowicz, C.M., 2006. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. Journal of Clinical Investigation 116, 146-155.

Zosky, G.R., Berry, L.J., Elliot, J.G., James, A.L., Gorman, S, and Hart, P.H., 2011. Vitamin D deficiency causes deficits in lung function and alters lung structure. American Journal of Respiratory and Critical Care Medicine 183, 1336-1343.

Key facts

- Periodontitis is an inflammatory disease of the supporting tissues of the teeth, caused by microorganisms, resulting in progressive destruction of the periodontal ligament and bone loss.
- Data on the prevalence of periodontal disease show that 5% to 20% of any population suffers from generalized severe periodontitis.
- Periodontitis requires treatment and may be costly and uncomfortable for the patient.
- Vitamin D supplementation reduced tooth loss and alveolar ridge resorption.
- There are valid reasons to assume that vitamin D plays a significant role in reducing the risk of periodontitis.

Summary points

- The pathogenesis of periodontal disease involves activation of host-defense cells by bacterial release of inflammatory mediators, which results in the destruction of connective tissue and alveolar bone.
- Vitamin D increases calcium absorption and stimulates osteoblasts to enable normal bone growth and preservation.
- Vitamin D may be also beneficial for the treatment of periodontal disease, because of its anti-inflammatory effect through inhibition of cytokine production and stimulation of immune cells to secrete peptides with potent antibiotic activity.
- Vitamin D induces the formation of cathelicidin and other defensins that combat bacterial infection. Polymorphisms of the vitamin D receptor gene have been associated with periodontitis.
- The research to date suggests the beneficial effects of vitamin D on periodontal disease. Vitamin D supplementation regimen may positively affect periodontal disease, above and beyond standard periodontal care; however, further studies are needed.

14. Vitamin D and periodontal disease

N. Garcia[1,2], D. Miley[1,2] and D.A. Dixon[2]
[1]Saint Louis University Center for Advanced Dental Education, 3320 Rutger Street, St. Louis, MO 63104, USA; [2]Department of Applied Dental Medicine, Southern Illinois University School of Dental Medicine, 2800 College Avenue, Alton, IL 62002, USA; garciamn@slu.edu

Abstract

Periodontal disease is an inflammatory condition, characterized by alveolar bone loss induced by the host immune response to bacterial attack. Because of vitamin D is associated with some inflammatory diseases and plays a significant role in bone homeostasis and immunity, vitamin D deficiency could negatively affect the periodontium and increase tooth loss. In spite of the limitations of previous studies, there are valid reasons to assume that vitamin D plays a significant role in reducing the risk of periodontitis. Vitamin D promotes calcium absorption and protects bone strength; the active form of vitamin D, 1α,25-dihydroxyvitamin, function as an immunomodulator because of its anti-inflammatory and antimicrobial properties that can inhibit the inflammatory response through modulation of cytokine production by immune cells and stimulation of monocytes and macrophages to secrete peptides with potent antibacterial action; another potential function of vitamin D in periodontal health is supported by the finding that polymorphisms of the vitamin D receptor gene are associated with periodontitis, clinical attachment loss, bone loss, and/or tooth loss. These multiple actions of vitamin D are potentially interesting for the treatment of patients with periodontitis. If increased vitamin D intake can reduce alveolar bone loss, oral vitamin D supplementation could prove to be a safe, effective, low-cost adjunctive systemic/pharmacologic therapy for the prevention and treatment of periodontal disease. The research to date suggests the beneficial effects of vitamin D on periodontal disease; however, further studies are needed.

Keywords: periodontitis prevention and treatment, alveolar bone, tooth loss

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_14, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
PMN	Polymorphonuclear leukocyte
VDR	Vitamin D receptor

14.1 Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth, caused by specific microorganisms, where bacterial biofilms adhere to the gingival tissue leading to a host response and resulting in progressive destruction of the periodontal ligament and alveolar bone (Newman *et al.*, 2011). According to the Research, Science and Therapy committee of the American Academy of Periodontology (2005), data on the prevalence of periodontal disease show that 5% to 20% of any population suffers from generalized severe periodontitis. Mild or moderate forms of the disease affect the majority of all adults.

Tooth loss is the result of lack of support from bony and connective tissues, and periodontitis is the primary cause of this condition. Even though there has been a decrease in edentulism (total tooth loss) during the last several years, almost one out of every four individuals over 65 have lost all of their teeth. This results in negative esthetic and functional consequences that affect speech and chewing capacity (Bailey *et al.*, 2005). Edentulism reduces the quality of life, affects nutrition, and requires costly treatments. Specific factors responsible for alveolar bone loss and continued resorption of the residual alveolar ridge (subsequent to tooth loss) are not well understood, nor have treatments to prevent alveolar bone loss been universally successful.

Any form of periodontitis requires treatment and may be costly and uncomfortable for the patient. In many cases tooth loss is inevitable, requiring far more complex and expensive options. Reducing periodontal disease-induced oral bone loss would lessen the need for periodontal surgical treatments, reduce tooth loss, and improve the outcome of interventions, such as tooth implants, when they cannot be avoided.

14.2 Periodontal disease pathogenesis and the relationship with vitamin D

The pathogenesis of periodontal disease involves a bacteria-host interaction, with microorganisms and their byproducts inducing tissue destruction indirectly by activating host defense cells including lymphocytes, macrophages and PMNs, which in turn produce and release mediators that result in connective tissue breakdown and alveolar bone resorption (Page and Kornman, 1997).

As part of this process, components of microbial plaque have the capacity to induce the initial infiltrate of inflammatory cells including lymphocytes, macrophages, and PMNs. Bacterial components, especially lipopolysaccharide, have the capacity to activate macrophages to synthesize and secrete different molecules like cytokines, cell proteins that are the cause of lymphocytic infiltration, that result in bone resorption and dissolution of the extracellular matrix. Cytokines regulate the body's inflammatory response by transmitting signals between cells. The contribution of interleukin-1, interleukin-6 and tumor necrosis factor-α to periodontal tissue destruction, is associated with the resorption of alveolar bone because of their potent osteoclastogenic features (Graves and Cochran, 2003; Offenbacher, 1996). Interleukin-1 also stimulates the release of metalloproteinases which degrade the extracellular matrix and prostaglandin E2 which is responsible for vasodilatation, edema, and bone loss. Antigen-stimulated lymphocytes (B and T cells) are also important in this process. These locally produced cascade of events finally lead to connective tissue breakdown and bone loss via activation of osteoclast-mediated bone resorption (Cochran, 2008).

It has been suggested that vitamin D and proinflammatory cytokines as well as periodontal disease are associated with diabetes mellitus and cardiovascular diseases (Grossi and Genko, 1998; Page, 1998; Zitterman, 2003). These conditions are also linked with obesity and because vitamin D and 25(OH)D are stored in adipose tissue, obese subjects have low serum levels of 25(OH)D. Moreover, specific vitamin D-receptor genotypes have been shown to be associated with localized aggressive periodontal disease, oral bone loss, clinical attachment loss, and tooth loss (Hildebolt, 2005).

14.3 The effect of vitamin D on oral health

Many studies have investigated the effect of vitamin D on various aspects of oral health. Krall *et al.* (2001) examined tooth loss in elderly patients. During the first three years of their study, 27% of the placebo group (no supplementation) lost one or more teeth. Only 13% of the subjects in the supplement group (500 mg calcium and 700 IU cholecalciferol) lost one or more teeth. Since the supplement group also received calcium, it is not possible to determine the sole effect of vitamin D on these subjects.

Dietrich *et al.* (2004) evaluated whether serum 25-hydroxyvitamin D concentrations were associated with periodontal disease in the third National Health and Nutrition Examination Survey. They analyzed data on attachment loss, which is the clinical diagnostic criteria for periodontal disease. They found that 25(OH)D concentrations were significantly and inversely associated with attachment loss in men and women ages 50 years and older.

Calcium and vitamin D supplementation may have an effect of alveolar ridge resorption. 60 subjects who received immediate dentures were given either placebos or 750 mg calcium and 375 IU vitamin D per day. Bone loss was measured with panoramic radiographs over a one year period. Subjects that completed the study and received the supplementation had 36% less

bone loss than subjects taking the placebo (Wical and Brussee, 1979). Calcium and vitamin D supplementation has also been shown to increase mandibular bone mass. As part of a study on estrogen/hormone replacement therapy, Hildebolt *et al.* (2004) found that 17 of 19 women who took calcium and vitamin D supplementation for 3 years had increases in mandibular bone mass.

A recent study (Jabbar *et al.*, 2011) examined women with osteoporosis. Their subjects with osteoporosis had lower 25(OH)D levels. Women with osteoporosis also exhibited a greater incidence of periodontal disease. They concluded that 25(OH)D levels were significantly lower in subjects with either past or active periodontal disease. Periodontal disease was best predicted by vitamin D status along with receptor activator of nuclear factor kappa-B ligand, serum C-terminal telopeptide and weight. Vitamin D insufficiency has also been associated with maternal periodontal disease in pregnancy. Boggess *et al.* (2011) examined the relationship between maternal vitamin D status and periodontal disease. They concluded that the adjusted odds ratio for moderate to severe periodontal disease among women with vitamin D insufficiency was 2.1.

Not all studies are in agreement. Liu *et al.* (2009) examined patients with chronic periodontitis and aggressive periodontitis. They conducted full periodontal examinations and determined plasma calcifediol levels. These levels were significantly higher in the patients with aggressive periodontitis as compared to healthy controls. The plasma calcifediol levels were not significantly different between patient with chronic periodontitis and healthy controls. The levels of calcifediol were also significantly correlated with the bleeding index in the aggressive periodontitis patients. It is important to note that the authors indicate that nearly 60% of their subjects had lower levels of plasma calcifediol when considering 27 nmol/l as the lower limit of the normal hematic range, therefore, most of these subjects were vitamin D deficient to begin with. This may explain the contradictory results from this study.

14.4 The potential benefit of vitamin D on periodontal disease

Only a few studies have evaluated the function of vitamin D on periodontal disease. Some information has been published using the Third National Health and Nutrition Examination Survey data to analyze the association between serum 25(OH)D levels and attachment loss and gingival inflammation (Dietrich *et al.*, 2004, 2005). These epidemiological studies found more attachment loss and gingival inflammation in subjects with lower the levels of 25(OH)D. These associations were independent of factors such as smoking and diabetes. While useful, these cross-sectional, survey type studies do not provide any significant insights on possible physiologic mechanisms of the potential protective effect of vitamin D on periodontal disease, because no analyses of local bacterial and cytokine production were performed.

14.4.1 Effects of vitamin D supplementation on periodontal disease

The effects of vitamin D supplementation on periodontal disease have not been totally elucidated. Some early reports suggested that vitamin D supplementation reduced tooth loss and alveolar ridge resorption (Baxter, 1984; Habets *et al.*, 1988a,b; Krall *et al.*, 2001; Renner *et al.*, 1984; Wical and Brussee, 1979; Wical and Swoope, 1974), but most of these studies did not directly assess periodontal disease status.

The available experience on dietary supplementation is not very extensive but hopeful. A two-year program of dietary supplementation with 400 IU per day of vitamin D and increased calcium intake to 1000 mg per day resulted in either maintenance or an increase of mandibular bone mass in 83% of a study population (Kribbs, 1992). Unfortunately, data on periodontal disease status was not given and 20% of these patients were edentulous. The results of this study are comparable to those that were reported by Civitelli *et al.* (2002), in three-year hormone replacement study, where women who received 1000 mg of calcium and 400 IU of vitamin D per day showed significant increases in both alveolar crest height and alveolar bone mass. Women in this study were, however, periodontally healthy and there was no control group in which women received only a placebo.

A recent survey to determine the frequency of vitamin D and calcium oral supplementation use by patients in periodontal maintenance programs at two dental schools in Missouri and Illinois, found that only 7% had vitamin D and calcium levels that met the Food and Nutrition Board's recommended intakes (Dixon *et al.*, 2009). These results suggest that even though the use of vitamin D and calcium supplementation has been widely promoted for years, the knowledge of the benefits of supplementation needs to be better disseminated and research needs to continue in order to determine optimal levels of supplementation.

A cross-sectional pilot study which followed the survey analysis, evaluated the differences in alveolar crest heights between subjects who were taking calcium and vitamin D supplementation and those who were taking neither vitamin D nor calcium supplements and had dietary intakes of calcium <1000 mg/day and of vitamin D <400 IU/day (non-takers). All eight clinical parameters of periodontal health were better at baseline in subjects who took supplementation relative to non-takers, (Miley *et al.*, 2009). A second goal of this study was to determine the changes in periodontal disease measurements for a one year period. The results supported the concept that a regular vitamin D supplementation regimen may positively affect periodontal disease, above and beyond standard periodontal care, despite the small number of subjects and the fact that the participants' periodontal health was generally good and the intakes of vitamin D were relatively low (which may have reduced the potential benefits from vitamin D and calcium supplementation) (Garcia *et al.*, 2011).

A recent case-control study, examined the relationship between vitamin D status and periodontal disease among pregnant women, measuring serum 25(OH)D levels. Despite some limitations, this study suggested that vitamin D supplementation could improve maternal oral health (Boggess

et al., 2011). According to the authors, the relationship between maternal vitamin D status, periodontal disease, and adverse pregnancy outcomes requires further studies before definitive conclusions could be stated. However, the data suggested that improvement of vitamin D status is a potential intervention to improve oral health among pregnant women.

In summary, all of the currently available studies on vitamin D supplementation and oral health have some methodological weaknesses. Since both vitamin D and calcium were used in most of the previous studies, it is difficult to distinguish between the effects of the two supplements. For the effects of vitamin D on periodontitis, there has never been a clinical trial in which randomization and masking were carefully controlled, the periodontal disease and serum 25(OH)D status of patients were known, and periodontal disease measures were the primary outcomes. Therefore, there is sufficient observational and preliminary evidence supporting the rationale for a prospective, controlled evaluation of the effects of oral supplementation on periodontal disease.

14.4.2 Mechanisms to explain the role of vitamin D in reducing the risk of periodontitis

In spite of the limitations of previous studies, there are valid reasons to assume that vitamin D plays a significant role in reducing the risk of periodontitis. First, it is well known that vitamin D increases calcium absorption and protects bone strength. Second, vitamin D has anti-inflammatory properties that can inhibit the inflammatory response associated with periodontal disease. Third, vitamin D induces the formation of cathelicidin and other defensins that combat bacterial infection. Fourth, polymorphisms of the VDR gene have been associated with periodontitis, alveolar bone loss, and clinical attachment loss.

Vitamin D's anti-inflammatory properties

Besides its role in bone and calcium homeostasis, nonskeletal (autocrine/paracrine) actions of vitamin D have been described. The biologically-active hormonal form of vitamin D [1,25-dihydroxyvitamin D] is a potent immunomodulator (De luca, 2004, Penna *et al.*, 2005). In this form, $1,25(OH)_2D$ inhibits T cell proliferation, alters cytokine production by Th1 lymphocytes, and is a powerful inhibitor of B cell-mediated antibody production and dendritic cell differentiation, while it stimulates macrophage phagocytosis of bacteria (Arnson *et al.*, 2007; Boonstra *et al.*, 2001; Mattner *et al.*, 2000; Rigby *et al.*, 1987; Van Etten and Mathieu, 2005). It can also modulate monocyte and macrophage differentiation and the release of inflammatory cytokines and chemokines (Abu-Amer and Bar-Shavit, 1993, 1994; Arnson *et al.*, 2007; Cohen *et al.*, 1986; Helming *et al.*, 2005; Hewison *et al.*, 2004). Therefore, it is reasonable to expect that local production of $1,25(OH)_2D$ can inhibit the inflammatory response associated with periodontal disease and could result in a lowering of blood levels of C-reactive protein, which is the most widely used biomarker of inflammation (Paraskevas *et al.*, 2008, Ridker and Silvertown, 2008). Dietrich *et al.* (2005) provide some evidence of the anti-inflammatory properties of vitamin D. They examined 77,503 gingival units (teeth) in 6,700 non-smoking subjects. Compared with sites in subjects in the lowest 25(OH)D quintile, sites in subjects in the highest 25(OH)D quintile were

20% less likely to bleed upon gingival probing. This is significant due to the fact that bleeding upon probing is the best measure of clinical gingival inflammation.

Stimulation of defensins and cathelicidins

One of the mechanisms that is proposed to explain the role of vitamin D in reducing the risk of periodontal disease, is the stimulation of α- and β-defensins and cathelicidins, proteins with broad-spectrum antimicrobial activity (Cannell *et al.*, 2008). Beyond vitamin D's anti-inflammatory effect, monocytes and macrophages exposed to lipopolysaccharide or *Mycobacterium tuberculosis* up-regulate the VDR gene and the 25-hydroxyvitamin D-1α-hydroxylase gene (which converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D). Increased production of 1,25-dihydroxyvitamin D results in the synthesis of cathelicidin, a peptide capable of destroying *M. tuberculosis* as well as other infectious agents (Liu *et al.*, 2006). Vitamin D is thus capable of stimulating the production of a potent natural antibiotic. When serum levels of 25-hydroxyvitamin D fall below 20 ng per milliliter (50 nmol per liter), the monocyte or macrophage is prevented from initiating the innate immune response (Liu *et al.*, 2006). This concept may explain why groups who are vitamin D-deficient tend to have a more aggressive form of tuberculosis and are more prone to contracting the disease. This, in turn, suggests that differences in serum 25-hydroxyvitamin D levels may contribute to the susceptibility to microbial infections (Liu *et al.*, 2006).

Vitamin D receptors

The importance of vitamin D's role in periodontal disease, is supported as well by findings that suggest genetic polymorphisms of the VDR gene are associated with periodontitis, alveolar bone loss, clinical attachment loss, and/or tooth loss (Inagaki *et al.*, 2003; Yoshie *et al.*, 2007). VDRs are found in nearly every cell of the body. Recently, $1,25(OH)_2D3$ has been shown to have anticancer, immune modulatory and cardiovascular regulator effects by activating the VDR, a transcription factor of the nuclear receptor superfamily. The $1,25(OH)_2D3$-VDR system plays a role in maintaining oral health through its effects on bone and mineral metabolism and innate immunity, and its dysfunction have been reported to be associated with periodontal disease (Amano *et al.*, 2009). Deng *et al.* (2011) conducted a meta-analysis to investigate the association of VDR gene polymorphisms with susceptibility to aggressive and chronic periodontitis. Their analysis of 15 studies included 1,338 cases and 1,302 controls and confirmed that the VDR gene is a candidate gene for periodontitis.

14.5 The potential impact of vitamin D on periodontal disease treatment

The standard of care for periodontal disease treatment is mostly local (meticulous oral hygiene and scaling and root planing). Although scrupulous oral hygiene has long been accepted as a successful method for preventing and treating periodontal disease, patients are often not able to maintain these practices. Scaling and root planing and surgical options (root debridement,

resective and regenerative procedures) are well-accepted treatments for periodontal disease. However, many patients do not have access to, cannot afford, or cannot tolerate the discomfort associated with such therapies. Systemic/antimicrobial and pharmacologic treatments overcome some of these restrictions, but there are limitations associated with most of these options. Oral vitamin D supplementation could prove to be a safe, effective, low-cost adjunctive systemic/pharmacologic therapy for the prevention and treatment of periodontal disease.

If increased vitamin D and calcium intake can demonstrate reduced alveolar bone loss, oral vitamin D supplementation could prove to be a safe, effective, low-cost adjunctive systemic/pharmacologic therapy, resulting in a significant impact on the quality of life by leading to: (1) a reduction in surgical treatments aimed at repairing or minimizing alveolar bone damage caused by periodontal disease; (2) a decrease in tooth loss attributable to alveolar bone loss; (3) a decline in the placement of implants or prosthetic devices (bridges and partial dentures) to replace lost teeth; and (4) a reduction in the number of denture remakes and relines required because of alveolar bone resorption.

Vitamin D supplementation could also impact periodontal disease treatment outcomes. Bashutski *et al.* (2011) evaluated the vitamin D status and the results from periodontal surgery. They found improved clinical attachment level gain and probing depth reduction for patients that had sufficient vitamin D levels prior to surgery. Patients that had insufficient vitamin D levels prior to surgical treatment lost clinical attachment after treatment. This patient population all exhibited severe periodontal disease. Vitamin D supplementation could prove to be a valuable adjunctive treatment for those requiring extensive surgical procedures.

14.6 Conclusions

Vitamin D is antibacterial, anti-inflammatory, bone protective, safe, inexpensive, available over the counter and easy to use. The research to date suggests the beneficial effects of vitamin D on periodontal disease; however, further studies are needed. There is no randomized controlled trial on vitamin D's effect on alveolar bone loss in periodontal disease. Vitamin D supplementation in deficient patients could potentially result in less non-surgical and surgical periodontal treatment, less tooth loss, fewer denture remakes and relines and fewer dental implant placements. This dietary supplement could have a major impact on periodontal disease prevention and treatment.

References

Abu-Amer, Y. and Bar-Shavit, Z.,1993. Impaired bone marrow-derived macrophage differentiation in vitamin D deficiency. Cellular Immunology 151, 356-368.

Abu-Amer, Y. and Bar-Shavit, Z., 1994. Regulation of TNF-alpha release from bone marrow-derived macrophages by vitamin D [erratum appears in J Cell Biochem 1994 56(3), 26]. Journal of Cellular Biochemistry 55, 435-444.

Amano, Y., Komiyama, K. and Mikishima, M., 2009. Vitamin D and periodontal disease. Journal of Oral Science 51, 11-20.

American Academy of Periodontology: Research, Science and Therapy Committee, 2005. Epidemiology of periodontal diseases. Journal of periodontology 76, 1406-1419.

Arnson, Y., Amital, H. and Shoenfeld, Y., 2007. Vitamin D and autoimmunity: new etiological and therapeutic considerations. Annals of the Rheumatic Diseases 66, 1137-1142.

Bashutski, J., Eber, R., Kinney, J., Benavides, E., Maitra, S., Braun, T., Giannobile, W. and McCauley, L. 2011. The impact of vitamin D status on periodontal surgery outcomes. Journal of Dental Research 90, 1007-1012.

Bailey, R., Gueldner, S., Ledikwe, J. and Smiciklas-Wright, H., 2005. The oral health of older adults: an interdisciplinary mandate. Journal Gerontological Nursing 31(7), 11-17.

Baxter, J.C., 1984. The nutritional intake of geriatric patients with varied dentitions. Journal of Prosthetic Dentistry 51, 164-168.

Boggess, K.A., Espinola, J.A., Moss, K., Beck, J., Offenbacher, S. and Camargo Jr., C.A., 2011. Vitamin D status and periodontal disease among pregnant women. Journal of Periodontology 82, 195-200.

Boonstra, A., Barrat, F.J., Crain, C., Heath, V.L., Savelkoul, H.F. and O'Garra, A., 2001. 1alpha,25-dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. Journal of Immunology 167, 4974-4980.

Cannell, J.J., Vieth, R., Willett, W., Zasloff, M., Hathcock, J.N., White, J.H., Tanumihardjo, S.A., Larson-Meyer, D.E., Bischoff-Ferrari, H.A., Lamberg-Allardt, C., Lappe, J.M., Norman, A.W., Zittermann, A., Whiting, S.J., Grant, D.A., Hollis, B.W. and Giovannucci, E., 2008. Cod liver oil, vitamin A toxicity, frequent respiratory infections, and the vitamin D defiency epidemic. Annals of Otology, Rhinology and Laryngology 117, 864-870.

Civitelli, R., Pilgram, T.K., Dotson, M., Muckerman, J., Lewandowski, N., Armamento-Villareal, R., Yokoyama-Crothers, N., Kardaris, E.E., Hauser, J., Cohen, S. and Hildebolt, C.F., 2002 Hormone/estrogen replacement therapy improves alveolar and postcranial bone density in postmenopausal women. Archives of Internal Medicine.162, 1409-1415.

Cochran, D.L., 2008 Inflammation and bone loss in periodontal disease. Journal of Periodontology 79, 1569-1576.

Cohen, M.S., Mesler, D.E., Snipes, R.G. and Gray, T.K., 1986. 1,25-Dihydroxyvitamin D3 activates secretion of hydrogen peroxide by human monocytes. Journal of Immunology 136, 1049-1053.

DeLuca, H.F., 2004. Overview of general physiologic feactures and functions of vitamin D. American Journal of Clinical Nutrition 80 (6 suppl), 1689s-1696s.

Deng, H., Liu, F., Pan, Y., Jin, X., Wang, H., Cao, J. 2011. BsmI, TaqI, ApaI, and FokI polymorphisms in the vitamin D receptor gene and periodontitis: a meta-analysis of 15 studies including 1338 cases and 1302 controls. Journal of Clinical Periodontology 38, 199-207.

Dietrich, T., Joshipura, K.J., Dawson-Hughes, B., Bischoff-Ferrari, H.A. 2004. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. American Journal of Clinical Nutrition 80, 108-113.

Dietrich, T., Nunn, M., Dawson-Hughes, B., Bischoff-Ferrari, H. A. 2005. Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation. American Journal of Clinical Nutrition 82, 575-580.

Dixon, D., Hildebolt, C.F., Miley, D.D., Garcia, M.N., Pilgram, T.K., Couture, R.A., Anderson Spearie, C. and Civitelli, R., 2009 Calcium and vitamin D use among adults in periodontal-disease maintenance programs. British Dental Journal 206, 627-631.

Garcia, M.N., Hildebolt, C.F., Miley, D.D., Couture, R.A., Dixon, D., Anderson Spearie, C., Langenwalter, E.M., Shannon, W., Deych, E., Mueller, C. and Civitelli, R., 2011. One-year effects of vitamin D and calcium supplementation on chronic periodontitis. Journal of Periodontology 82, 25-32.

Graves, D.T. and Cochran, D., 2003. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. Journal of Periodontololology 74, 391-401.

Grossi, S.G. and Genco, R.J., 1988. Periodontal disease and diabetes mellitus: a two-way relationship. Annals of Periodontology 3, 51-61.

Habets, L.L., Bras, J. and Borgmeyer-Hoelen, A.M. 1988a. Mandibular atrophy and metabolic bone loss. Endocrinology, radiology and histomorphometry. International Journal of Oral Maxillofacial Surgery 17, 208-211.

Habets, L.L., Bras, J. and Van Merkesteyn, J.P., 1988b. Mandibular atrophy and metabolic bone loss. Histomorphometry of iliac crest biopsies in 74 patients. International Journal of Oral Maxillofacial Surgery 17, 325-329.

Helming, L., Bose, J., Ehrchen, J., Schiebe, S., Frahm, T., Geffers, R., Probst-Kepper, M., Balling, R. and Lengeling, A., 2005 1alpha,25-dihydroxyvitamin D3 is a potent suppressor of interferon gamma-mediated macrophage activation. Blood 106, 4351-4358.

Hewison, M., Zehnder, D., Chakraverty, R. and Adams, J.S., 2004. Vitamin D and barrier function: a novel role for extra-renal 1 alpha-hydroxylase. Molecular & Cellular Endocrinology 215, 31-38.

Hildebolt, C., 2005 Effect of vitamin D and calcium on periodontitis. Journal of Periodontology 76, 1576-1587.

Hildebolt, C.F., Pilgram, T.K., Dotson, M., Armamento-Villareal, R., Hauser, J., Cohen, S. and Civitelli, R., 2004. Estrogen and/or calcium plus vitamin D increase bone mass. Journal of Periodontology 75, 811-816.

Inagaki, K., Krall, E.A., Fleet, J.C. and Garcia, R.I., 2003. Vitamin D receptor alleles, periodontal disease progression, and tooth loss in the VA dental longitudinal study. Journal of Periodontology 74, 161-167.

Jabbar, S., Drury, J., Fordham, J., Datta, H.K., Francis, R.M. and Tuck, S.P., 2011. Plasma vitamin D and cytokines in periodontal disease and postmenopausal osteoporosis. Journal of Periodontal Research 46, 97-104.

Krall, E., Wehler, C., Garcia, R., Harris, S. and Dawson-Hughes, B., 2001. Calcium and vitamin D supplements reduce tooth loss in the elderly. 2001a. The American Journal of Medicine 111, 452-456.

Kribbs, P.J., 1992. Two-year changes in mandibular bone mass in an osteoporotic population. Journal of Prosthetic Dentistry 67, 653-655.

Liu, K., Meng, H., Tang, X., Xu, L., Zhang, L., Chen, Z., Shi, D., Feng, X. and Lu, R., 2009. Elevated plasma calcifediol is associated with aggressive periodontitis. Journal of Periodontology 80, 1114-1120.

Liu, P.T., Stenger, S., Li, H., Wenze, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., Kamen, D.L., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R.L., Eisenberg, D., Hewison, M., Hollis, B.W., Adams, J.S., Bloom, B.R. and Modlin, R.L., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311, 1770-1773.

Mattner, F., Smiroldo, S., Galbiati, F., Muller, M., Di Lucia, P., Poliani, P.L., Martino, G., Panina-Bordignon, P. and Adorini, L., 2000. Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D(3). European Journal of Immunology 30, 498-508.

Miley, D.D., Garcia, M.N., Hildebolt, C.F., Shannon, W., Couture, R.A., Anderson Spearie, C., Dixon, D., Langenwalter, E.M., Mueller, C. and Civitelli, R., 2009 Cross-sectional study of the effects of vitamin D and calcium oral supplementation on Periodontitis. Journal of Periodontology 80, 1433-1439.

Newman, G., Takei, H., Klokkevold, P. and Carranza, F., 2011. Carranza's clinical periodontology. Elsevier publishers, Philadelphia, PA, USA, pp 41-42.

Offenbacher, S., 1996. Periodontal diseases: pathogenesis. Annals of Periodontology 1, 821-878.

Page, R.C. and Kornman, K.S., 1997. The pathogenesis of human periodontitis: an introduction. Periodontology 14, 9-11.

Page, R.C., 1998. The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. Annals of Periodontology 3, 108-120.

Paraskevas, S., Huizinga, J.D. and Loos, B.G., 2008. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. Journal of Clinical Periodontology 35, 277-290.

Penna, G., Roncari, A., Amuchastegui, S., Daniel, K.C., Berti, E., Colonna, M. and Adorini, L., 2005. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood 106(10), 3490-3497.

Renner, R.P., Boucher, L.J. and Kaufman, H.W., 1984. Osteoporosis in postmenopausal women. J Prosthet Dent 52, 581-528.

Rigby, W.F., Denome, S. and Fanger, M.W., 1987. Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D3. Specific inhibition at the level of messenger RNA. Journal of Clinical Investigation 79, 1659-1664.

Ridker, P.M. and Silvertown, J.D., 2008 Inflammation, C-reactive protein, and atherothrombosis. Journal of Periodontology 79, 1544-1551.

Van Etten, E. and Mathieu, C., 2005. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. Journal of Steroid Biochemistry & Molecular Biology 97, 93-101.

Wical, K.E. and Brussee, P., 1979. Effects of a calcium and vitamin D supplement on alveolar ridge resorption in immediate denture patients. Journal of Prosthetic Dentistry 41, 4-11.

Wical, K.E. and Swoope, C.C., 1974. Studies of residual ridge resorption. II. The relationship of dietary calcium and phosphorus to residual ridge resorption. Journal of Prosthetic Dentistry 32, 13-22.

Yoshie, H., Kobayashi, T., Tai, K. and Galicia, J., 2007. The role of genetic polymorphism in periodontitis. Periodontology 43, 102-132.

Zittermann, A., 2003. Vitamin D in preventive medicine: are we ignoring the evidence? British Journal of Nutrition 89, 552-572.

Key facts

- The prevalence of vitamin D insufficiency in cystic fibrosis (CF) is very high but only recently the topic has received increased attention.
- Bisphosphonates improve bone mineral density (BMD) in CF patients. Due to a lack of adequate studies it remains unclear whether vitamin D supplementation has a positive effect on BMD.
- Despite the lack of evidence, the current European and US guidelines for vitamin D supplementation recommend relatively high doses of colecalciferol and aim for s25(OH)D>50 nmol/l (Europe) and s25(OH)D>75 nmol/l (US) in all CF patients.
- Ultraviolet lamps have high theoretical potential in successfully improving vitamin D status in CF patients, but this alternative and its safety have been addressed by a limited number of studies.
- Because of its immunomodulatory and glucose-lowering effects, vitamin D may have a broad potential in CF patients, who often suffer from chronic inflammation and CF-related diabetes. Caution should however be exercised as dampened inflammation may not only be of benefit.

Summary points

- CF is a common hereditary disease; CF patients suffer mainly from recurrent lung infections and defective food nutrient uptake.
- Many patients with CF are deficient in vitamin D, despite daily vitamin supplementation.
- There are several known reasons behind the tendency for low vitamin D levels in CF.
- Vitamin D insufficiency is associated with bone disease in CF, but it has not been clearly proven that supplementation improves bone health in CF.
- The US guidelines for vitamin D supplementation in CF published in 2005 were shown to be ineffective at improving vitamin D status of the patients. The latest European (2011) and US (2012) guidelines are mainly consensus based due to lack of evidence and low number of relevant studies available.
- Vitamin D levels in CF patients exhibit strong seasonality pattern, and use of ultraviolet lamps might be an alternative to vitamin D supplementation in patients who are willing to stay adherent with the therapy.
- Test tube experiments and studies describing CF populations have recently created hypotheses that vitamin D may have beneficial effect on the CF patients' immunity, lung function and blood glucose regulation.
- There is need for well-performed vitamin D supplementation trials to show whether the hypotheses are true and whether vitamin D is beneficial or harmful to CF patients.
- There are a few registered current/future trials that might answer some of these questions, but more studies are clearly needed.

15. Vitamin D and cystic fibrosis

T. Pincikova[1] and M. Flodström-Tullberg[2]
[1]Stockholm CF Center, Karolinska Institutet, Karolinska University Hospital Huddinge, 14186 Stockholm, Sweden; [2]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital Huddinge, 14186 Stockholm, Sweden;
terezia.pincikova@ki.se

Abstract

Cystic fibrosis (CF) patients suffer from malnutrition and lung infections, both acute and chronic. The majority are also vitamin D insufficient despite vitamin D supplementation. With better medical care the life expectancy for CF patients has increased and so has the prevalence of long-term complications, such as bone disease and CF-related diabetes. Vitamin D insufficiency is associated with bone disease in CF, but evidence that vitamin D supplementation improves bone mineral density in CF patients is missing. The pathophysiology of vitamin D insufficiency in CF is multifactorial. Serum 25-hydroxyvitamin D (s25(OH)D) levels in CF patients fluctuate throughout the year, depending on the amount of UV exposure, with lowest values at the end of the winter. The current European and US guidelines for vitamin D supplementation for CF patients prefer colecalciferol over ergocalciferol, consider age of the patients, endorse individualisation of the supplementation regimen and are mostly consensus based due to lack of CF-specific evidence. In all age groups, s25(OH)D >50 nmol/l (Europe) or s25(OH)D >75 nmol/l (US) should be achieved and maintained. The current literature is missing CF-specific hard evidence on many issues, such as the optimal s25(OH)D level, the relative benefit of colecalciferol vs. ergocalciferol and daily vs. weekly supplementation regimen. The use of UV lamps is a promising alternative, but has so far been ineffective in raising s25(OH)D level in CF patients due to poor adherence with the treatment. *In vitro* experiments and human descriptive studies indicate that vitamin D might have beneficial immunomodulatory and glucose-lowering effects in CF patients, but a careful approach is to be recommended given the absence of well-designed intervention trials proving causality, safety and clinical relevance of the amplitude of the potential effects. There are a few registered trials currently running or planned to start soon, which address some of these questions.

Keywords: bone mineral density, guidelines, immunomodulation, CF-related diabetes, registered trials

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_15, © Wageningen Academic Publishers 2013

Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
BMD	Bone mineral density
CF	Cystic fibrosis
CFRD	Cystic fibrosis-related diabetes
CFTR	Cystic fibrosis transmembrane regulator
FEV1	Forced expiratory volume in 1 second per cent predicted
IgG	Immunoglobulin G
LPS	Lipopolysaccharide
RCT	Randomised controlled trial
s25(OH)D	Serum 25-hydroxyvitamin D
Th	T helper
UVB	Ultraviolet B
VDR	Vitamin D receptor

15.1 Cystic fibrosis

CF is the most prevalent life-shortening disease with autosomal-recessive heredity pattern in Caucasians (FitzSimmons, 1993). More than 1,500 disease-causing mutations have been described so far. The most common mutation is F508del. The *cftr* gene encodes a chloride channel located in the membranes of epithelial cells. Mutations in the *cftr* lead to abnormally thick mucus and dysfunction of epithelial organs. Thus, symptoms of the disease mainly come from the pulmonary and gastrointestinal tracts, including the liver and pancreas.

The major underlying reason for morbidity and premature death is the progressive pulmonary disease. CF patients have an increased overall infection susceptibility and particularly enhanced susceptibility to contracting chronic lung *Pseudomonas aeruginosa* infection/colonisation. Moreover, some CF patients develop ABPA, i.e. lung colonisation with *Aspergillus* species and allergic reactions directed against the fungi. The chronic infection/colonisation and recurrent exacerbations are associated with chronic inflammation and a decline in lung function. The majority of the CF patients suffer from pancreatic insufficiency, resulting in malabsorption and insufficiency of fat soluble vitamins. Therefore, pancreatic enzyme preparations have to be taken with every meal by patients who are pancreatic insufficient, and fat soluble vitamin supplements are recommended for all CF patients (Peters and Rolles, 1993).

The therapy available today is symptomatic and requires a multifaceted approach given that multiple organs are affected. The main focus of the therapy is strict infection control and tight nutritional follow-up. Advances in the CF disease management have been increasing the life-span of the CF patients worldwide, and it has been calculated that 95% of the CF patients born in Scandinavia today will live longer than 25 years (Hjelte, 2008). However, this has led to increased prevalence of long-term complications of the disease, such as CFRD and CF-related bone disease,

and the focus of the therapy has slightly shifted towards these complications. Sadly enough, the potential causative therapy for the majority of the CF patients still mainly remains at the research level, and the only available treatment of the end-stage pulmonary disease is lung transplantation.

15.2 Cystic fibrosis-related low bone mineral density and vitamin D

With improved medical care and increased life expectancy of patients with CF, bone disease has become a common complication (Aris *et al.*, 2005). The prevalence of low BMD and its complications in CF is high. Observational studies show pooled prevalence of osteoporosis and osteopenia in adults with CF being as high as 23.5% and 38.0%, respectively (Paccou *et al.*, 2010). Even adolescents with CF were shown to have decreased total body BMD and cortical wrist BMD (Buntain *et al.*, 2004), and some studies report low BMD also in young children with CF (Sermet-Gaudelus *et al.*, 2007).

The underlying reasons for poor bone health in CF include pancreatic insufficiency leading to malabsorption and low BMI, delayed puberty, glucocorticoid use (Aris *et al.*, 1996), immunosuppression after lung transplantation (Spira *et al.*, 2000), CFTR dysfunction (Le Heron *et al.*, 2010), the systemic response to infection (Shead *et al.*, 2010), vitamin K insufficiency (Nicolaidou *et al.*, 2006), compromised calcium balance (Schulze *et al.*, 2003), CFRD (Levin *et al.*, 1976) and reduced daily physical activity (Tejero-Garcia *et al.*, 2011). Vitamin D insufficiency has been associated with poor bone health (Stead *et al.*, 1988, Shane *et al.*, 1996). PTH correlates with s25(OH)D (West *et al.*, 2011) and it responds to vitamin D administration in CF (Brown *et al.*, 2003; Khazai *et al.*, 2009).

The complications of low BMD in CF include vertebral and non-vertebral fractures with a high prevalence (Paccou *et al.*, 2010), and severe kyphosis (Aris *et al.*, 1998), which can secondarily lead to severe morbidity with diminished lung function (O'Reilly *et al.*, 2009; Legroux-Gerot *et al.*, 2012) and deformity (Elkin *et al.*, 2001). In the long run, this may accelerate the progression of CF disease; therefore, the maintenance of bone health in CF patients is crucial.

Current prevention and treatment recommendations for CF-related low BMD are primarily based on targeting the known risk factors for poor bone health in CF (Aris *et al.*, 2005; Sermet-Gaudelus *et al.*, 2011; UK CF Trust, 2007). With vitamin D insufficiency being one of the observed risk factors associated with poor bone health in CF (Shane *et al.*, 1996; Stead *et al.*, 1988) the UK CF Trust, the European CF guidelines and the US Cystic Fibrosis Foundation Consensus Panel recommend routine vitamin D supplementation for CF patients (Aris *et al.*, 2005; Sermet-Gaudelus *et al.*, 2011; Tangpricha *et al.*, 2012; UK CF Trust, 2007). Secondarily, in patients with very low BMD (T/Z score ≤-2.0), with history of fragility fractures or after lung transplantation, bisphosphonates should be considered, but owing to several potential safety concerns and common adverse events their broader use cannot be recommended (Aris *et al.*, 2005; Sermet-Gaudelus *et al.*, 2011; UK CF Trust, 2007).

Interestingly, while oral and intravenous bisphosphonates have been proven to increase BMD in CF patients (Conwell and Chang, 2009), no relevant studies have managed to prove that vitamin D supplementation improves BMD (Ferguson and Chang, 2009). Whether the lack of hard evidence for vitamin D supplementation improving BMD is the reason why vitamin D supplementation in CF received little attention in the past remains unclear.

15.3 Vitamin D insufficiency in cystic fibrosis

Due to lack of attention to vitamin D in CF in the past, patients used to be given multi-vitamin supplements in uniform dose and s25(OH)D level would not be monitored (Hahn *et al.*, 1979). As a consequence, vitamin D insufficiency has been a common phenomenon among both the paediatric and adult CF patients over the last few decades (Boyle *et al.*, 2005; Donovan *et al.*, 1998; Hanly *et al.*, 1985; Henderson and Lester, 1997; Khazai *et al.*, 2009; Pincikova *et al.*, 2011a; Shane *et al.*, 1996; Solomons *et al.*, 1981; Stead *et al.*, 1988; Stephenson *et al.*, 2007; Wolfenden *et al.*, 2008,); despite routine vitamin supplementation, vitamin D and/or its metabolites studied in various CF patient populations were lower than in control non-CF subjects (Congden *et al.*, 1981; Hahn *et al.*, 1979; Reiter *et al.*, 1985; Rovner *et al.*, 2007). Interestingly, one third of CF infants detected by newborn screening were reported to have low s25(OH)D level at initial evaluation (Sokol *et al.*, 1989), implying that vitamin D insufficiency occurs already early in life in CF patients. Luckily, the data on vitamin D concentrations suggest a trend toward higher s25(OH)D levels over recent years, which may well reflect somewhat improved attention to vitamin D by CF centres (Hall *et al.*, 2010). Indeed, the frequency of s25(OH)D measurements in patients followed at the Stockholm CF Centre increased steadily during years 2007-2009, which complies with the suggested hypothesis that the attention to vitamin D in the field of CF is continuously improving (Pincikova and Hjelte, 2010).

The underlying reasons for the high prevalence of vitamin D insufficiency in CF include pancreatic exocrine insufficiency, little body fat, low serum vitamin D binding protein (DBP) concentrations (Coppenhaver *et al.*, 1981), impaired absorption of vitamin D2 despite pancreatic enzyme supplementation and hypothesised accelerated excretion of vitamin D before hepatic hydroxylation of the vitamin (Lark *et al.*, 2001), liver disease causing impaired hepatic hydroxylation (Wills and Savory, 1984), low sunlight exposure (Thompson, 1987), and low adherence with vitamin D supplement prescriptions (Hollander *et al.*, 2010). Several studies have highlighted sun exposure as an important determinant of s25(OH)D levels in CF patients. Moreover, s25(OH)D values were shown to exhibit a clear season-dependent pattern (Pincikova and Hjelte, 2010; Robberecht *et al.*, 2011; Thompson, 1987). Robberecht *et al.* (2011) showed that s25(OH)D concentrations are significantly higher during 'Months with high UVB exposure' (May-October) than during 'Months with low UVB exposure' (November-April). This confirmed the results of our retrospective study of Stockholm CF patients (Pincikova and Hjelte, 2010). In line with this, the vitamin D supplementation recommendations published very recently by the CFF recommend the yearly screening for vitamin D status to be done preferably at the end of winter (Tangpricha *et al.*, 2012).

15.4 Current vitamin D supplementation guidelines in cystic fibrosis and future challenges

Because vitamin D in CF has only recently received attention, the current recommendations for vitamin D supplementation for CF-related bone disease are ranked as 'C' (UK CF Trust, 2007), due to absence of directly applicable studies of good quality (Petrie *et al.*, 1995). The Cochrane Cystic Fibrosis and Genetic Disorders Group assessed the content of the Intervention Review 'Vitamin D supplementation for cystic fibrosis' as up-to-date in August 2011. Here, the authors concluded that there is no evidence of benefit or harm for vitamin D supplementation in CF and suggested to consider adherence to relevant CF guidelines before further evidence appears (Ferguson and Chang, 2009).

The current UK CF Trust guidelines are based on the Report of the UK CF Trust Bone Mineralisation Working Group published in February 2007. The report recommends daily doses of ergocalciferol or colecalciferol of 400 IU for infants, 400-800 IU for 1-12 years old patients, 800-2,000 IU for patients older than 12 years (UK CF Trust, 2007). The US CFF consensus panel recommends ergocalciferol due to low cost, higher safety and wide variety of dosing alternatives, as compared with colecalciferol. The recommended routine dosing here is 400 IU for infants and 800 IU for patients older than 1 year of age, and if this supplementation fails to achieve or maintain optimal s25(OH)D levels, a high-dose ergocalciferol repletion regimen should be initiated, where the unresponsive individuals are administered 50,000 IU ergocalciferol weekly for 8 weeks. The patients who still fail to achieve optimal s25(OH)D levels should be given 50,000 IU ergocalciferol biweekly for 8 weeks (Aris *et al.*, 2005). Both the UK and the US guidelines endorse individualisation of the vitamin D supplementation, aiming for s25(OH)D levels 75-150 nmol/l. Here, the maximum recommended dosing is 50,000 IU biweekly for patients ≥5 years in the US guidelines (Aris *et al.*, 2005), whereas the UK guidelines do not specify maximum dosing (UK CF Trust, 2007). Moreover, both of these guidelines underline that some patients may require aggressive supplementation with higher dosing, and they recommend even more polar vitamin D analogues (calcitriol, alfacalcideol or calcifediol) or phototherapy in cases where the patients do not respond adequately to more aggressive supplementation with ergocalciferol or colecalciferol. Furthermore, they highlight the need for additional research to optimise dosing and choice of the proper vitamin D analogue, and to retest the response to intramuscular ergocalciferol, which might be another potential way of vitamin D supplementation in nonresponsive patients despite that the single study done in this area has failed to prove it (Ontjes *et al.*, 2000).

Comparing these guidelines with the vitamin D intake recommendations for the healthy, non-CF, population, they endorse somewhat higher vitamin D dosing for routine supplementation (IOM, 2010). In addition, they are very flexible, recommending an individualised approach to dosing and to the choice of vitamin D analogue. Thus, one would expect these guidelines to be sufficient and well-working. Therefore, it is striking that the majority of CF patients worldwide continue to have insufficient s25(OH)D levels (Boyle *et al.*, 2005; Khazai *et al.*, 2009; Pincikova *et al.*, 2011a; Stephenson *et al.*, 2007; Wolfenden *et al.*, 2008). This might be partly caused by low patient adherence with vitamin prescriptions (Hollander *et al.*, 2010), but a number of studies have found

the currently recommended vitamin D supplement doses in CF ineffective. Already in year 1985 it was shown that supplementation with 800 IU per day in adolescents and adults with CF was unsuccessful in raising s25(OH)D levels (Hanly *et al.*, 1985). Kelly *et al.* (2002) demonstrated that 95% of CF patients needed to be administered 1,800 IU ergocalciferol daily in order to achieve the target s25(OH)D level 50 nmol/l, implying that the dose of 800 IU is inadequate. The recommended high-dose aggressive repletion regimen with oral ergocalciferol endorsed in the US recommendations for individuals unresponsive to the routine dosing has been tested in a study by Boyle *et al.* (2005). They reported that treatment with 50,000 IU ergocalciferol weekly for 8 weeks resulted in 8% patients achieving optimal s25(OH)D levels, and that none of those patients who went on to receive 50,000 IU ergocalciferol biweekly for 8 weeks corrected their vitamin D insufficiency. Haworth *et al.* (2004) evaluated the effect of two pills Calcichew D3 Forte per day (1000 mg calcium and 800 IU colecalciferol daily) compared with placebo, in CF patients with low BMD, all of them getting their standard ergocalciferol dose of 900 IU per day. Here again, there was no change in s25(OH)D levels between the treatment and the placebo group after 1 year of intervention (Haworth *et al.*, 2004). Clearly, the guidelines developed by UK CF Trust in 2007 and by the US Cystic Fibrosis Foundation consensus panel in 2005 seem to be inadequate. The first study showing that achieving s25(OH)D levels 75 nmol/l in all treated subjects is possible was published in 2009, where 100% of the adult CF patients who ingested 50,000 IU of vitamin D3 weekly for 3 months reached s25(OH)D levels above 75 nmol/l. Patients who received 50,000 IU of vitamin D2 weekly for 3 months also increased their s25(OH)D significantly at 3 months compared with baseline, and 60% of them reached s25(OH)D >75 nmol/l (Khazai *et al.*, 2009).

Collectively, the studies and observations reviewed above highlighted that the recommendations for prevention and treatment of CF-related bone disease clearly needed an update. Thus, EuroCareCF funded a work package in order to develop the recently published European CF bone mineralisation guidelines (Sermet-Gaudelus *et al.*, 2011). These guidelines recommend higher doses of vitamin D than the older US or UK guidelines, but on the other hand they did not find enough support in the CF-specific literature for the benefit and safety of s25(OH)D levels >75 nmol/l in the CF population. Thus, they recommend s25(OH)D levels above the threshold 50 nmol/l. Vitamin D2 or D3 should be administered in the starting dose of 1000-2,000 IU/day in infants and 1000-5,000 IU/day in patients above 1 year of age. The dose should later be adjusted individually in order to keep s25(OH)D level above the threshold of 50 nmol/l (Sermet-Gaudelus *et al.*, 2011). The publishing of the updated European guidelines was followed by the development of new guidelines for the management of vitamin D deficiency in CF patients by the CFF Vitamin D Evidence-Based Review Committee, which were published online on March 7 this year (Tangpricha *et al.*, 2012). Here again, the committee had to provide consensus recommendations on most of the issues because of very limited CF-specific evidence. In brief, the committee recommends colecalciferol over ergocalciferol, the dosage from previous recommendations (Aris *et al.*, 2005) has been increased, age and season are given consideration and the recommended s25(OH)D level is 75-125 nmol/l for all age groups (Tangpricha *et al.*, 2012). Whether application of the recently published European guidelines (Sermet-Gaudelus *et al.*, 2011) and the just published CFF guidelines (Tangpricha *et al.*, 2012) leads to better vitamin D status in CF patients remains to be evaluated.

Another important issue that remains to be solved is the choice of the most proper first-hand vitamin D analogue. Due to lack of relevant CF-specific studies in the area at the time when the US (2005) or UK (2007) guidelines were structured, the recommendation of the first hand 'analogue-of-choice' was based on local circumstances such as availability of the various vitamin D analogues on the local market, price and dosing possibilities (Aris *et al.*, 2005; UK CF Trust, 2007). This may however decrease the possibility for individualised dosing and aggressive supplementation regimens. However, already in 2001 it was demonstrated that ergocalciferol is absorbed less efficiently in CF than in control subjects (Lark *et al.*, 2001), and more recently, Khazai *et al.* (2009) showed that high-dose colecalciferol treatment was more efficacious than high-dose ergocalciferol treatment in raising s25(OH)D levels in 30 adult CF patients, where 100% of patients treated with colecalciferol reached s25(OH)D level of 75 nmol/l, whereas only 60% of patients treated with ergocalciferol in the same dose achieved these concentrations. Not surprisingly, this finding in the CF population confirms findings in various non-CF populations, where high-dose colecalciferol was more effective than high-dose ergocalciferol in raising s25(OH) D levels (Armas *et al.*, 2004; Glendenning *et al.*, 2009; Leventis and Kiely, 2009; Romagnoli *et al.*, 2008; Trang *et al.*, 1998). Thus, the recently published European guidelines favour colecalciferol over ergocalciferol (Sermet-Gaudelus *et al.*, 2011).

Yet it has to be noted that the study performed in CF patients by Khazai *et al.* (2009) demonstrated that there was a significant decrease in PTH from baseline to final measurements in both the colecalciferol and ergocalciferol treatment groups and there was no significant difference in the change in PTH concentrations between the two treatment groups. A similar finding was obtained in non-CF population where despite significant difference in achieved s25(OH)D levels, no difference in PTH concentrations was noted, comparing groups treated with ergocalciferol versus colecalciferol (Glendenning *et al.*, 2009). This questions the clinical importance of the difference in efficacy in solely raising s25(OH)D levels. Moreover, the results of the trial by Khazai *et al.* (2009) might have been confounded by the fact that the ergocalciferol contained an oil-based carrier and the colecalciferol capsules contained a powder-based carrier. The choice of carrier may be of relevance in the pancreatic-insufficient CF population suffering from fat malabsoption. In addition, several studies performed in non-CF subjects, to whom lower daily doses of supplemented vitamin D were administered, failed to show any difference between the efficacy of ergocalciferol and colecalciferol (Biancuzzo *et al.*, 2010; Gordon *et al.*, 2008; Holick *et al.*, 2008). Since low daily dosing rather than high-dose once-weekly dosing is the most common way of vitamin D supplementation in the majority of CF populations, these findings in non-CF populations might be of relevance. Thus, one could postulate that the suggestion that colecalciferol is of preference in the CF population is preliminary, underlining the need for more therapeutic trials in this area.

Last but not least, another question that remains unanswered is the optimal s25(OH)D level to be recommended for CF patients. The current European guidelines differ from the current US guidelines, endorsing a lower threshold of 50 nmol/l compared to the 75 nmol/l in the US guidelines (Aris *et al.*, 2005; Sermet-Gaudelus *et al.*, 2011; Tangpricha *et al.*, 2012). The lower threshold of s25(OH)D 75 nmol/l for optimal vitamin D status has been proposed (Holick,

2009) based on the finding of PTH levels beginning to plateau when s25(OH)D concentration is around 75 nmol/l (Chapuy *et al.*, 1997; Holick, 2005; Thomas *et al.*, 1998) and the s25(OH)D >75 nmol/l has been referred to as optimal even in relation to other endpoints (Bischoff-Ferrari *et al.*, 2006). However, the European experts interested in both CF and bone metabolism reached consensus on not recommending s25(OH)D >75 nmol/l for CF patients yet, because the long term consequences of these s25(OH)D levels have not been extensively explored (Sermet-Gaudelus *et al.*, 2011). They also referred to the study by Chapelon *et al.* (2009) who suggested that PTH levels in the high normal range may promote bone formation in children and reasoned that too low PTH levels may block bone formation. Indeed, persistently low intact PTH levels have recently been demonstrated to be independently related to aortic arch calcification and mortality in incident hemodialysis patients (Rhee *et al.*, 2012). Moreover, Malabanan *et al.* (1998) showed that healthy adults who had s25(OH)D >50 nmol/l had no significant change in their PTH level after receiving 50,000 IU of vitamin D once a week for 8 weeks, which indicates that the threshold of 75 nmol/l may be unnecessarily high. On the other hand, West *et al.* (2011) recently examined PTH concentrations as the function of s25(OH)D levels in CF patients and concluded that the optimal s25(OH)D levels in CF in relation to PTH are higher than those in healthy population, recommending s25(OH)D >87.5 nmol/l in CF. This might imply that s25(OH)D levels of 75 nmol/l recommended by the US CFF consensus panel do not keep PTH in CF patients at the very minimum, which questions the reasoning of the experts forming the European guidelines and their fear of blocking bone formation by too low PTH if patients get supplemented to reach levels of 75 nmol/l. Moreover, Heaney *et al.* (2003) reported calcium absorption from the gut to be 65% higher at s25(OH)D concentrations averaging 86.5 nmol/l than at s25(OH)D levels averaging 50 nmol/l. However, this study was done in non-CF postmenopausal women and studying calcium absorption in CF patients might give different results. Indeed, Hillman *et al.* (2008) studied true calcium absorption in children with CF and concluded that it was in the normal range at baseline. Treatment with 2,000 IU colecalciferol with or without 1g calcium for 6 months increased neither s25(OH)D nor true calcium absorption significantly. However, the study might have been underpowered and there was a slight trend towards increase in true calcium absorption in the group receiving both colecalciferol and calcium. With all of this information collectively, one could summarise that the issue of optimal s25(OH)D levels for preventing CF-related low BMD and for maximising calcium absorption in CF patients remains controversial, which highlights acute need for studies in this area.

Considering the problem of intestinal malabsorption in the vast majority of CF patients, an interesting issue which might be worth further exploration is the potential use of UVB radiation for increasing or maintaining s25(OH)D levels. A non-randomised trial (Gronowitz *et al.*, 2005), a case-series (Chandra *et al.*, 2007) and an RCT (Khazai *et al.*, 2009) showed that UVB radiation for 2 to 3 months does increase s25(OH)D significantly in CF patients who are adherent to the treatment. Unfortunately, all of these studies report low adherence to the UVB radiation treatment (Chandra *et al.*, 2007; Gronowitz *et al.*, 2005; Khazai *et al.*, 2009). Due to non-adherence, the RCT published by Khazai *et al.* (2009) failed to show any increase in s25(OH)D in patients receiving the UVB treatment, but after excluding the 4 non-adherent patients from the analysis, the rise in s25(OH)D was similar to the rise in s25(OH)D in patients receiving vitamin D2 *per os*. This

suggests that if one could make the CF patients adherent with the UVB treatment, the UVB may well become a successful treatment option for the vitamin D insufficiency in CF.

15.5 Extra-skeletal effects of vitamin D in cystic fibrosis

While the importance of vitamin D in maintaining bone health has been known for a long time, more recent studies have suggested that vitamin D also has extraskeletal effects. In 1979 and a few years after that it was discovered that several tissues and cells such as pancreas, skin, placenta, and immune cells express the VDR (Bhalla *et al.*, 1983; Stumpf *et al.*, 1979). Around the same time it was also found that the enzyme responsible for the second hydroxylation step in the production of active vitamin D, 1α-hydroxylase (Cyp27B), is expressed not only in the kidneys but also by other types of cells and tissues (Adams *et al.*, 1983; Weisman *et al.*, 1979). Collectively, these observations suggested that active vitamin D is not produced solely in the kidneys and that vitamin D might have biological functions besides maintaining calcium homeostasis. Today we know that vitamin D has an impact also on the skin, the cardiovascular system and the immune system, etc (recently reviewed in (Christakos and DeLuca, 2011; Schwalfenberg, 2011).

The immunomodulatory effects of vitamin D have received great attention lately as data suggests that vitamin D has important functions both in host defense and in dampening inflammation. By regulating the production of tight junction proteins vitamin D may contribute to the intactness (and thereby permeability) of epithelial barriers covering the skin, gut, urinary and respiratory tracts (Clairmont *et al.*, 1996; Gniadecki *et al.*, 1997; Palmer *et al.*, 2001). Vitamin D is also important for the production of antimicrobial peptides at such sites (Wang *et al.*, 2004), suggesting important functions in host defense. Low vitamin D levels have been associated with autoimmune and allergic diseases (Van Belle *et al.*, 2011). This, together with the observations that VDR and enzymes involved in the generation of biologically active vitamin D are expressed in numerous types of immune cells, clearly point to that vitamin D also has effects on the immune system. The immunomodulatory actions of vitamin D have recently been covered in excellent reviews (Baeke *et al.*, 2010; Van Belle *et al.*, 2011), and the emerging picture is that vitamin D stimulates the actions of cells associated with innate immunity (e.g. macrophages), but dampens the adaptive immune response at least in part by reducing the production of Th1 and Th17-associated cytokines (e.g. IL-2, IFNg, IL-17 and IL-21) and decreasing plasma cell differentiation and the production of IgM and IgG.

VDR is expressed in murine and human respiratory epithelial cells (Menezes *et al.*, 2008; Yim *et al.*, 2007; Zhong, *et al.*, 1994), and human lung epithelial cells can convert inactive 25-dihydroxyvitamin D3 to active 1,25-dihydroxyvitamin D3 (Hansdottir *et al.*, 2008). Human tracheobronchial epithelial cells express VDR-regulated genes including the antimicrobial peptide cathelicidin upon stimulation with 25-dihydroxyvitamin D3 (Hansdottir *et al.*, 2008), suggesting that vitamin D is produced locally in the lung and that epithelial cell derived vitamin D contributes to the host innate immune defense.

CF has been associated with increased inflammation as well as impaired ability to clear viral and bacterial infections. As reviewed above, CF is often associated with vitamin D deficiency or insufficiency (Boyle *et al.*, 2005; Khazai *et al.*, 2009; Pincikova *et al.*, 2011a; Stephenson *et al.*, 2007; Wolfenden *et al.*, 2008). Based upon the bulk of data supporting an important role for vitamin D in regulating immune- and epithelial cell functions more studies on the effect of vitamin D insufficiency on host defense and inflammation in CF is clearly warranted. Recent studies have demonstrated that human bronchial and tracheal cell lines harboring mutations in CFTR respond to active vitamin D by increasing the expression of cathelicidin mRNA levels (McNally *et al.*, 2011; Yim *et al.*, 2007). Moreover, the production of pro-inflammatory cytokines (Il-6 and IL-8) was reduced when such cells were pre-treated with active vitamin D prior to challenge with LPS or conditioned medium from cultures with *Pseudomonas aeruginosa* (McNally *et al.*, 2011). On top of that, heightened Th2 reactivity in cohort of CF patients with ABPA correlates with lower mean s25(OH)D, and *in vitro* addition of $1,25(OH)_2$-D3 substantially reduces dendritic cell expression of the costimulatory molecule OX40L, increases dendritic cells expression of TGF-beta, increases Treg TGF-beta expression and reduces Th2 responses by CD4+ T cell from CF patients with ABPA (Kreindler *et al.*, 2010). Collectively, these studies indicate that vitamin D may have a therapeutical potential in CF. Indeed, by stimulating the production of antimicrobial peptides it may promote antibacterial defense in the lung. At the same time it may dampen excessive inflammatory responses and prevent/treat ABPA. Clinical trials are clearly warranted to investigate the potentially beneficial effects of vitamin D supplementation on antimicrobial defense and inflammation in the CF lung.

These CF-specific *in vitro* findings and hypotheses they created are in agreement with our human epidemiological study results. We demonstrated that serum total IgG levels are negatively associated with s25(OH)D ($P<0.001$) and supplemented and total vitamin D intake per kilogram bodyweight ($P<0.001$ and $P=0.002$ respectively). Moreover, s25(OH)D is positively associated with FEV1 ($P=0.025$) in a large, well-described cohort of Scandinavian CF children and adults (n=896). This cross-sectional multi-centre study used robust multiple linear regression models, which enabled us to correct for potential confounders and explained 37.5-39.8% of variation in IgG and 30.8% of variation in FEV1 (Pincikova *et al.*, 2011a). A significant positive association between s25(OH)D and FEV1 ($R^2=0.30$, $P<0.001$) in a CF cohort was also described in a separate study. This study had a descriptive retrospective design and evaluated data from 185 adult CF patients collected form medical records over 2 years. Here, the use of specific vitamin D supplements provided protection against vitamin D insufficiency whereas use of multivitamins did not (Wolfenden *et al.*, 2008). On the other hand, Sagel *et al.* (2011) demonstrated that daily supplementation with AquADEKs® softgels over 3 months in fourteen CF patients does improve vitamin D status, albeit it does not completely normalise it. Moreover, they observed modest increase in both weight percentile and lung function (Sagel *et al.*, 2011).

Even post-transplant CF patients might be affected by the immunomodulatory function of vitamin D. In a cohort of 41 lung transplanted CF patients, the cumulative dose of hydrocortisone needed during the first week after transplantation was inversely related to their cumulative vitamin D supplement dose ($r=-0.52$; $P<0.05$). Additionally, $1,25(OH)_2D$ was positively correlated with

cumulative dose of cyclosporine A one year after transplantation (r=0.93; $P<0.01$) (Pincikova *et al.*, 2011b). This confirms findings in renal transplant recipients (Sato *et al.*, 2009), and suggests that awareness of vitamin D and its complex effects is to be recommended in order to optimise the management of lung transplanted CF patients.

Not only immunomodulatory effects but also glucose-lowering properties of vitamin D have been described. Several outstanding reviews cover this topic (e.g. Takiishi *et al.*, 2010). Clearly, vitamin D may very well play role in the pathogenesis of diabetes mellitus and have a therapeutic potential in the prevention and/or treatment of both type 1 diabetes and type 2 diabetes. The reported positive associations of s25(OH)D concentration with insulin secretion and peripheral insulin sensitivity in type 2 diabetes (Chiu *et al.*, 2004), and the reported ability of vitamin D to decrease the incidence of type 1 diabetes (Hyppönen *et al.*, 2001) may theoretically benefit CF patients as well. This hypothesis is supported by results of our cross-sectional multi-centre study where s25(OH)D <30 nmol/l (OR=1.79) and vitamin D insufficiency degree (OR=1.36) were significant independent risk factors for CFRD ($P<0.05$ in both) in 898 Scandinavian CF patients. Moreover, vitamin D status was associated with HbA_{1c} values, and the associations were stronger in CF children than in CF adults (Pincikova *et al.*, 2011c). Given the increasing prevalence of CFRD and its profound impact on CF patients' clinical status (Lanng *et al.*, 1992), one might hypothesise that the described associations between vitamin D and CFRD may well be clinically relevant. Thus, further studies testing the causality of vitamin D insufficiency and the amplitude of clinical benefit of vitamin D supplementation are strongly warranted.

All the findings mentioned above suggest beneficial effects of vitamin D for CF patients. However, a careful approach is to be recommended. The CF-specific findings are either demonstrated *in vitro* only, or they present pure associations without any evidence of causality. The general 'publication bias', with negative studies not being published easily has to be kept in mind. Moreover, the CF population differs from non-CF populations in many aspects. For instance, it is debated whether dampening the chronic lung inflammation in patients chronically infected with *P. aeruginosa* is beneficial in the long run (Elston and Geddes, 2007). Maybe the inflammation keeps the bacteria in check, preventing their spread and septicaemia? Maybe CF patients down-regulate vitamin D when needed, such as in some cases of infection when increased immune responses are required? Is aggressive vitamin D treatment in these cases not harmful? Adequately powered, well-designed long-term trials with hard endpoints are crucial before we say definitely yes to vitamin D for CF patients.

15.6 Ongoing research

The results of the research papers available today consistently suggest that improved attention to vitamin D in CF is likely to reduce morbidity through improved bone health, lung function, glucose homeostasis, and modulation of inflammation. However, the current true knowledge only allows us to create hypotheses. Before any conclusions about the benefit and safety of vitamin

D supplementation in CF patients can be drawn, we need to await the conclusions of the current and future clinical trials, RCTs being most important.

We searched for trials registered at Clintrials.gov and found five clinical trials exploring the effects of vitamin D supplementation *per os* in patients with CF, which have not yet published their results (Table 15.1). We hope that these registered trials together with other ongoing trials will succeed in answering the vitamin-D-and-CF-specific questions asked. Thereby, they will contribute each with their own unique piece to the puzzle and throw more light into this yet unexplored but relevant research area. Then maybe the 'vitamin D and CF'-mystery will once be solved.

Acknowledgements

T.P. and M.F-T. are supported by Karolinska Institutet, Stockholm, Sweden. Studies on Cystic Fibrosis in the M.F-T. laboratory are supported by The Swedish Cystic Fibrosis Association and Erica Lederhausen's Minnesstiftelse. Professor Lena Hjelte is acknowledged for consultation.

Table 15.1. Trials (unpublished) registered at clinicaltrials.gov under the identifier number studying vitamin D supplementation effects in cystic fibrosis patients.

	Identifier				
	NCT00685971	**NCT00788138**	**NCT01426256**	**NCT01321905**	**NCT01222273**
Trial design	double-blinded placebo-controlled RCT	double-blinded placebo-controlled RCT	double-blinded placebo-controlled RCT	open-label RCT	open-label non-randomised trial
Principal investigator	Stephenson	Tangpricha	Tangpricha	Hjelte	Pilewski
Study population	adult patients with s25(OH)D <75 nmol/l on stable vitamin D supplementation	adult patients admitted to hospital for acute lung exacerbation	patients older than 16 years	patients older than 5 years	patients older than 11 years with ABPA by *Aspergillus fumigatus*
Dose and analogue	5,000 IU per day colecalciferol	250,000 IU colecalciferol in single dose	250,000 IU (bolus dose) + 50,000 IU (maintenance dose every other week) colecalciferol	5,000 IU per day (if 6-15 years of age) or 7,150 IU per day (if older than 15 years) colecalciferol or ergocalciferol	4,000 IU per day colecalciferol
Study duration	12 weeks	3 months	12 months	3 months intervention followed by 2 months withdrawal	6 months
Primary outcome(s)	change in mean s25(OH)D	s25(OH)D and LL-37	composite number of deaths and re-hospitalisations	change in mean s25(OH)D	*Aspergillus*-induced IL-13 responses in CD4+ T-cells
Secondary outcomes	calcium, FEV1, PTH, creatinine, percentage of patients reaching s25(OH)D >75 nmol/l, subgroup analyses by pancreatic sufficiency vs. insufficiency	FEV1, days of hospitalisation, days on antibiotic therapy	inflammation, antimicrobial peptides, antibiotic use, FEV1, mortali-ty and re-hospitalisations as single measures	calcium, PTH, inflammation, infection, glucose tolerance, lung function, quality of life, adherence, safety	IgE and IgG, vitamin D levels, FEV1, cytokine production by *Aspergillus*-stimulated peripheral blood T-cells, urine/creatinine ratio

References

Adams, J.S., Sharma, O.P., Gacad, M.A. and Singer, F.R., 1983. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. The Journal of Clinical Investigation 72(5), 1856-1860.

Aris, R.M., Merkel, P.A., Bachrach, L.K., Borowitz, D.S., Boyle, M.P., Elkin, S.L., Guise, T.A., Hardin, D.S., Haworth, C.S., Holick, M.F., Joseph, P.M., O'Brien, K., Tullis, E., Watts, N.B. and White, T.B., 2005. Guide to bone health and disease in cystic fibrosis. The Journal of Clinical Endocrinology and Metabolism 90(3), 1888-1896.

Aris, R.M., Neuringer, I.P., Weiner, M.A., Egan, T.M. and Ontjes, D., 1996. Severe osteoporosis before and after lung transplantation. Chest 109(5), 1176-1183.

Aris, R.M., Renner, J.B., Winders, A.D., Buell, H.E., Riggs, D.B., Lester, G.E. and Ontjes, D.A., 1998. Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. Annals of Internal Medicine 128(3), 186-193.

Armas, L.A., Hollis, B.W. and Heaney, R.P., 2004. Vitamin D2 is much less effective than vitamin D3 in humans. The Journal of Clinical Endocrinology and Metabolism 89(11), 5387-5391.

Baeke, F., Takiishi, T., Korf, H., Gysemans, C. and Mathieu, C., 2010. Vitamin D: modulator of the immune system. Current Opinion in Pharmacology 10(4), 482-496.

Bhalla, A.K., Amento, E.P., Clemens, T.L., Holick, M.F. and Krane, S.M., 1983. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. The Journal of Clinical Endocrinology and Metabolism 57(6), 1308-1310.

Biancuzzo, R.M., Young, A., Bibuld, D., Cai, M.H., Winter, M.R., Klein, E.K., Ameri, A., Reitz, R., Salameh, W., Chen, T.C. and Holick, M.F., 2010. Fortification of orange juice with vitamin D(2) or vitamin D(3) is as effective as an oral supplement in maintaining vitamin D status in adults. The American Journal of Clinical Nutrition 91(6), 1621-1626.

Bischoff-Ferrari, H.A., Giovannucci, E., Willett, W.C., Dietrich, T. and Dawson-Hughes, B., 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. The American Journal of Clinical Nutrition 84(1), 18-28.

Boyle, M.P., Noschese, M.L., Watts, S.L., Davis, M.E., Stenner, S.E. and Lechtzin, N., 2005, Failure of high-dose ergocalciferol to correct vitamin D deficiency in adults with cystic fibrosis. American Journal of Respiratory and Critical Care Medicine 172(2), 212-217.

Brown, S.A., Ontjes, D.A., Lester, G.E., Lark, R.K., Hensler, M.B., Blackwood, A.D., Caminiti, M.J., Backlund, D.C. and Aris, R.M., 2003. Short-term calcitriol administration improves calcium homeostasis in adults with cystic fibrosis. Osteoporosis International 14(5), 442-449.

Buntain, H.M., Greer, R.M., Schluter, P.J., Wong, J.C., Batch, J.A., Potter, J.M., Lewindon, P.J., Powell, E., Wainwright, C.E. and Bell, S.C., 2004. Bone mineral density in Australian children, adolescents and adults with cystic fibrosis: a controlled cross sectional study. Thorax 59(2), 149-155.

Chandra, P., Wolfenden, L.L., Ziegler, T.R., Tian, J., Luo, M., Stecenko, A.A., Chen, T.C., Holick, M.F. and Tangpricha, V., 2007. Treatment of vitamin D deficiency with UV light in patients with malabsorption syndromes: a case series. Photodermatology, Photoimmunology and Photomedicine 23(5), 179-185.

Chapelon, E., Garabedian, M., Brousse, V., Souberbielle, J.C., Bresson, J.L. and de Montalembert, M., 2009. Osteopenia and vitamin D deficiency in children with sickle cell disease. Eurorean Journal of Haematology 83(6), 572-578.

Chapuy, M.C., Preziosi, P., Maamer, M., Arnaud, S., Galan, P., Hercberg, S. and Meunier, P.J., 1997. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporosis International 7(5), 439-443.

Chiu, K.C., Chu, A., Go, V.L. and Saad, M.F., 2004. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. The American Journal of Clinical Nutrition 79(5), 820-825.

Christakos, S. and DeLuca, H.F., 2011. Minireview: vitamin D: is there a role in extraskeletal health? Endocrinology 152(8), 2930-2936.

Clairmont, A., Tessman, D., Stock, A., Nicolai, S., Stahl, W. and Sies, H., 1996. Induction of gap junctional intercellular communication by vitamin D in human skin fibroblasts is dependent on the nuclear Induction of gap junctional intercellular communication by vitamin D in human skin fibroblasts is dependent on the nuclear vitamin D receptor. Carcinogenesis 17(6), 1389-1391.

Congden, P.J., Bruce, G., Rothburn, M.M., Clarke, P.C., Littlewood, J.M., Kelleher, J. and Losowsky, M.S., 1981. Vitamin status in treated patients with cystic fibrosis. Archives of Disease in Childhood 56(9), 708-714.

Conwell, L.S. and Chang, A.B., 2009. Bisphosphonates for osteoporosis in people with cystic fibrosis. Cochrane Database of Systematic Reviews (4), CD002010.

Coppenhaver, D., Kueppers, F., Schidlow, D., Bee, D., Isenburg, J.N., Barnett, D.R. and Bowman, B.H., 1981. Serum concentrations of vitamin D-binding protein (group-specific component) in cystic fibrosis. Human Genetics 57(4), 399-403.

Donovan Jr., D.S., Papadopoulos, A., Staron, R.B., Addesso, V., Schulman, L., McGregor, C., Cosman, F., Lindsay, R.L. and Shane, E., 1998. Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. American Journal of Respiratory and Critical Care Medicine 157(6 Pt 1), 1892-1899.

Elkin, S.L., Fairney, A., Burnett, S., Kemp, M., Kyd, P., Burgess, J., Compston, J.E. and Hodson, M.E., 2001. Vertebral deformities and low bone mineral density in adults with cystic fibrosis: a cross-sectional study. Osteoporosis International 12(5), 366-372.

Elston, C. and Geddes, D., 2007. Inflammation in cystic fibrosis – when and why? Friend or foe? Seminars in Respiratory and Critical Care Medicine 28(3), 286-294.

Ferguson, J.H. and Chang, A.B., 2009. Vitamin D supplementation for cystic fibrosis. Cochrane Database of Systematic Reviews (4), CD007298.

FitzSimmons, S.C., 1993. The changing epidemiology of cystic fibrosis. The Journal of Pediatrics 122(1), 1-9.

Glendenning, P., Chew, G.T., Seymour, H.M., Gillett, M.J., Goldswain, P.R., Inderjeeth, C.A., Vasikaran, S.D., Taranto, M., Musk, A.A. and Fraser, W.D., 2009. Serum 25-hydroxyvitamin D levels in vitamin D-insufficient hip fracture patients after supplementation with ergocalciferol and cholecalciferol. Bone 45(5), 870-875.

Gniadecki, R., Gajkowska, B. and Hansen, M., 1997. 1,25-dihydroxyvitamin D3 stimulates the assembly of adherens junctions in keratinocytes: involvement of protein kinase C. Endocrinology 138(6), 2241-2248.

Gordon, C.M., Williams, A.L., Feldman, H.A., May, J., Sinclair, L., Vasquez, A. and Cox, J.E., 2008. Treatment of hypovitaminosis D in infants and toddlers. The Journal of Clinical Endocrinology and Metabolism 93(7), 2716-2721.

Gronowitz, E., Larko, O., Gilljam, M., Hollsing, A., Lindblad, A., Mellstrom, D. and Strandvik, B., 2005. Ultraviolet B radiation improves serum levels of vitamin D in patients with cystic fibrosis. Acta Paediatrica 94(5), 547-552.

Hahn, T.J., Squires, A.E., Halstead, L.R. and Strominger, D.B., 1979. Reduced serum 25-hydroxyvitamin D concentration and disordered mineral metabolism in patients with cystic fibrosis. The Journal of Pediatrics 94(1), 38-42.

Hall, W.B., Sparks, A.A. and Aris, R.M., 2010. Vitamin D deficiency in cystic fibrosis. International Journal of Endocrinology 2010, 218691.

Hanly, J.G., McKenna, M.J., Quigley, C., Freaney, R., Muldowney, F.P. and FitzGerald, M.X., 1985. Hypovitaminosis D and response to supplementation in older patients with cystic fibrosis. The Quarterly Journal of Medicine 56(219), 377-385.

Hansdottir, S., Monick, M.M., Hinde, S.L., Lovan, N., Look, D.C. and Hunninghake, G.W., 2008. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. Journal of Immunology 181(10), 7090-7099.

Haworth, C.S., Jones, A.M., Adams, J.E., Selby, P.L. and Webb, A.K., 2004. Randomised double blind placebo controlled trial investigating the effect of calcium and vitamin D supplementation on bone mineral density and bone metabolism in adult patients with cystic fibrosis. Journal of Cystic Fibrosis 3(4), 233-236.

Heaney, R.P., Dowell, M.S., Hale, C.A. and Bendich, A., 2003. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. Journal of the American College of Nutrition 22(2), 142-146.

Henderson, R.C. and Lester, G., 1997. Vitamin D levels in children with cystic fibrosis. Southern Medical Journal 90(4), 378-383.

Hillman, L.S., Cassidy, J.T., Popescu, M.F., Hewett, J.E., Kyger, J. and Robertson, J.D., 2008. Percent true calcium absorption, mineral metabolism, and bone mineralization in children with cystic fibrosis: effect of supplementation with vitamin D and calcium. Pediatric Pulmonology 43(8), 772-780.

Hjelte, L., 2008. Cystisk fibros. In: Lindberg, T. and Lagerkrantz, H (ed.) Barnmedicin. Narayana Press, Denmark, pp. 484-491.

Holick M.F., 2005. Variations in 25-hydroxyvitamin D assay results. J Clin Endocrinol Metab (Letter to the Editor); 90(50), 210.

Holick, M.F., 2009. Vitamin D status: measurement, interpretation, and clinical application. Annals of Epidemiology 19(2), 73-78.

Holick, M.F., Biancuzzo, R.M., Chen, T.C., Klein, E.K., Young, A., Bibuld, D., Reitz, R., Salameh, W., Ameri, A. and Tannenbaum, A.D., 2008. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. The Journal of Clinical Endocrinology and Metabolism 93(3), 677-681.

Hollander, F.M., de Roos, N.M., Dopheide, J., Hoekstra, T. and van Berkhout, F.T., 2010. Self-reported use of vitamins and other nutritional supplements in adult patients with cystic fibrosis. Is daily practice in concordance with recommendations? International Journal for Vitamin and Nutrition Research 80(6), 408-415.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R. and Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358(9292), 1500-1503.

Institute of Medicine (IOM), 2010. Dietary reference intakes for calcium and vitamin D. Available at: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/DRI-Values.aspx.

Kelly, E., Marsh, R., Pencharz, P., Tullis, E., 2002. Effect of vitamin D supplementation on low serum 25-hydroxyvitamin D in adults with cystic fibrosis [Abstract]. Pediatr Pulmonol 24(Suppl), 344.

Khazai, N.B., Judd, S.E., Jeng, L., Wolfenden, L.L., Stecenko, A., Ziegler, T.R. and Tangpricha, V., 2009. Treatment and prevention of vitamin D insufficiency in cystic fibrosis patients: comparative efficacy of ergocalciferol, cholecalciferol, and UV light. The Journal of Clinical Endocrinology and Metabolism 94(6), 2037-2043.

Kreindler, J.L., Steele, C., Nguyen, N., Chan, Y.R., Pilewski, J.M., Alcorn, J.F., Vyas, Y.M., Aujla, S.J., Finelli, P., Blanchard, M., Zeigler, S.F., Logar, A., Hartigan, E., Kurs-Lasky, M., Rockette, H., Ray, A. and Kolls, J.K., 2010. Vitamin D3 attenuates Th2 responses to *Aspergillus fumigatus* mounted by CD4+ T cells from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. The Journal of Clinical Investigation 120(9), 3242-3254.

Lanng, S., Thorsteinsson, B., Nerup, J. and Koch, C., 1992. Influence of the development of diabetes mellitus on clinical status in patients with cystic fibrosis. European Journal of Pediatrics 151(9), 684-687.

Lark, R.K., Lester, G.E., Ontjes, D.A., Blackwood, A.D., Hollis, B.W., Hensler, M.M. and Aris, R.M., 2001. Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients. The American Journal of Clinical Nutrition 73(3), 602-606.

Le Heron, L., Guillaume, C., Velard, F., Braux, J., Touqui, L., Moriceau, S., Sermet-Gaudelus, I., Laurent-Maquin, D. and Jacquot, J., 2010. Cystic fibrosis transmembrane conductance regulator (CFTR) regulates the production of osteoprotegerin (OPG) and prostaglandin (PG) E2 in human bone. Journal of Cystic Fibrosis 9(1), 69-72.

Legroux-Gerot, I., Leroy, S., Prudhomme, C., Perez, T., Flipo, R.M., Wallaert, B. and Cortet, B., 2012. Bone loss in adults with cystic fibrosis: prevalence, associated factors, and usefulness of biological markers. Joint Bone Spine 79(1), 73-77.

Leventis, P. and Kiely, P.D., 2009. The tolerability and biochemical effects of high-dose bolus vitamin D2 and D3 supplementation in patients with vitamin D insufficiency. Scandinavian Journal of Rheumatoogy 38(2), 149-153.

Levin, M.E., Boisseau, V.C. and Avioli, L.V., 1976. Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. New England Journal of Medicine 294(5), 241-245.

Malabanan, A.O., Turner, A.K., Holick, M.F., 1998. Severe generalized bone pain and osteoporosis in a premenopausal black female: effect of vitamin D replacement. J Clin Densitometr 1, 201-204.

McNally, P., Coughlan, C., Bergsson, G., Doyle, M., Taggart, C., Adorini, L., Uskokovic, M.R., El-Nazir, B., Murphy, P., Greally, P., Greene, C.M. and McElvaney, N.G., 2011. Vitamin D receptor agonists inhibit pro-inflammatory cytokine production from the respiratory epithelium in cystic fibrosis. Journal Cystic Fibrosis 10(6), 428-434.

Menezes, R.J., Cheney, R.T., Husain, A., Tretiakova, M., Loewen, G., Johnson, C.S., Jayaprakash, V., Moysich, K.B., Salgia, R. and Reid, M.E., 2008. Vitamin D receptor expression in normal, premalignant, and malignant human lung tissue. Cancer Epidemiology, Biomarkers and Prevention 17(5), 1104-1110.

Nicolaidou, P., Stavrinadis, I., Loukou, I., Papadopoulou, A., Georgouli, H., Douros, K., Priftis, K.N., Gourgiotis, D., Matsinos, Y.G. and Doudounakis, S., 2006. The effect of vitamin K supplementation on biochemical markers of bone formation in children and adolescents with cystic fibrosis. European Journal of Pediatrics 165(8), 540-545.

Ontjes, D.A., Lark, R.K., Lester, G.E., Aris, R.M., 2000. Vitamin D depletion and replacement in patients with cystic fibrosis. In: Norman, A. and Bouillon, R. (eds.) Vitamin D endocrine system: structural, biological, genetic and clinical aspects. Thomasset, University of California, Riverside, CA, USA, 893-896.

O'Reilly, R., Fitzpatrick, P., Leen, G., Elnazir, B. and Greally, P., 2009. Severe bone demineralisation is associated with higher mortality in children with cystic fibrosis. Irish Medical Journal 102(2), 47-49.

Paccou, J., Zeboulon, N., Combescure, C., Gossec, L. and Cortet, B., 2010. The prevalence of osteoporosis, osteopenia, and fractures among adults with cystic fibrosis: a systematic literature review with meta-analysis. Calcified Tissue International 86(1), 1-7.

Palmer, H.G., Gonzalez-Sancho, J.M., Espada, J., Berciano, M.T., Puig, I., Baulida, J., Quintanilla, M., Cano, A., de Herreros, A.G., Lafarga, M. and Munoz, A., 2001. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. The Journal of Cell Biology 154(2), 369-387.

Peters, S.A. and Rolles, C.J., 1993. Vitamin therapy in cystic fibrosis – a review and rationale. Journal of Clinical Pharmacy and Therapeutics 18(1), 33-38.

Petrie, G.J., Barnwell, E., Grimshaw, J., on behalf of the Scottish Intercollegiate Guidelines Network, 1995. Clinical guidelines: criteria for appraisal for national use. Royal College of Physicians, Edinburgh, UK.

Pincikova, T. and Hjelte, L., 2010. Vitamin D Status in Stockholm Cystic Fibrosis Patients in 2007-2009. Journal of Cystic Fibrosis 9, Suppl 1. Abstract nr 353, S91.

Pincikova, T., Mared, L., Hjelte, L., 2011b. Interaction of fat-soluble vitamins with immunosuppressant drugs in lung transplanted CF patients. Journal of Cystic Fibrosis 10, Suppl 1. Abstract nr 306, S77.

Pincikova, T., Nilsson, K., Moen, I.E., Fluge, G., Hollsing, A., Knudsen, P.K., Lindblad, A., Mared, L., Pressler, T. and Hjelte, L., 2011c. Vitamin D deficiency as a risk factor for cystic fibrosis-related diabetes in the Scandinavian Cystic Fibrosis Nutritional Study. Diabetologia 54(12), 3007-3015.

Pincikova, T., Nilsson, K., Moen, I.E., Karpati, F., Fluge, G., Hollsing, A., Knudsen, P.K., Lindblad, A., Mared, L., Pressler, T. and Hjelte, L., 2011a. Inverse relation between vitamin D and serum total immunoglobulin G in the Scandinavian Cystic Fibrosis Nutritional Study. European Journal of Clinical Nutrition 65(1), 102-109.

Reiter, E.O., Brugman, S.M., Pike, J.W., Pitt, M., Dokoh, S., Haussler, M.R., Gerstle, R.S. and Taussig, L.M., 1985. Vitamin D metabolites in adolescents and young adults with cystic fibrosis: effects of sun and season. The Journal of Pediatrics 106(1), 21-26.

Rhee, H., Song, S.H., Kwak, I.S., Lee, S.B., Lee, D.W., Seong, E.Y. and Kim, I.Y., 2012. Persistently low intact parathyroid hormone levels predict a progression of aortic arch calcification in incident hemodialysis patients. Clinical and Experimental Nephrology [Epub ahead of print] PMID:22218932.

Robberecht, E., Vandewalle, S., Wehlou, C., Kaufman, J.M. and De Schepper, J., 2011. Sunlight is an important determinant of vitamin D serum concentrations in cystic fibrosis. European Journal of Clinical Nutrition 65(5), 574-579.

Romagnoli, E., Mascia, M.L., Cipriani, C., Fassino, V., Mazzei, F., D'Erasmo, E., Carnevale, V., Scillitani, A. and Minisola, S., 2008. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. The Journal of Clinical Endocrinology and Metabolism 93(8), 3015-3020.

Rovner, A.J., Stallings, V.A., Schall, J.I., Leonard, M.B. and Zemel, B.S., 2007. Vitamin D insufficiency in children, adolescents, and young adults with cystic fibrosis despite routine oral supplementation. The American Journal of Clinical Nutrition 86(6), 1694-1699.

Sagel, S.D., Sontag, M.K., Anthony, M.M., Emmett, P. and Papas, K.A., 2011. Effect of an antioxidant-rich multivitamin supplement in cystic fibrosis. Journal of Cystic Fibrosis 10(1), 31-36.

Sato, T., Fukagawa, M., Uchida, K., Katayama, A., Nagasaka, T., Matsuoka, S., Goto, N., Tominaga, Y., Kobayashi, T. and Nakao, A., 2009. 1,25-dihydroxyvitamin D synthesis after renal transplantation: the role of fibroblast growth factor 23 and cyclosporine. Clinical Transplantation 23(3), 368-374.

Schulze, K.J., O'Brien K, O., Germain-Lee, E.L., Baer, D.J., Leonard, A.L. and Rosenstein, B.J., 2003. Endogenous fecal losses of calcium compromise calcium balance in pancreatic-insufficient girls with cystic fibrosis. The Journal of Pediatrics 143(6), 765-771.

Schwalfenberg, G.K., 2011. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Molecular Nutrition and Food Research 55(1), 96-108.

Sermet-Gaudelus, I., Bianchi, M.L., Garabedian, M., Aris, R.M., Morton, A., Hardin, D.S., Elkin, S.L., Compston, J.E., Conway, S.P., Castanet, M., Wolfe, S. and Haworth, C.S., 2011. European cystic fibrosis bone mineralisation guidelines. Journal of Cystic Fibrosis 10 Suppl 2, S16-23.

Sermet-Gaudelus, I., Souberbielle, J.C., Ruiz, J.C., Vrielynck, S., Heuillon, B., Azhar, I., Cazenave, A., Lawson-Body, E., Chedevergne, F. and Lenoir, G., 2007. Low bone mineral density in young children with cystic fibrosis. American Journal of Respiratory and Critical Care Medicine 175(9), 951-957.

Shane, E., Silverberg, S.J., Donovan, D., Papadopoulos, A., Staron, R.B., Addesso, V., Jorgesen, B., McGregor, C. and Schulman, L., 1996. Osteoporosis in lung transplantation candidates with end-stage pulmonary disease. The American Journal of Medicine 101(3), 262-269.

Shead, E.F., Haworth, C.S., Barker, H., Bilton, D. and Compston, J.E., 2010. Osteoclast function, bone turnover and inflammatory cytokines during infective exacerbations of cystic fibrosis. Journal of Cystic Fibrosis 9(2), 93-98.

Sokol, R.J., Reardon, M.C., Accurso, F.J., Stall, C., Narkewicz, M., Abman, S.H. and Hammond, K.B., 1989. Fat-soluble-vitamin status during the first year of life in infants with cystic fibrosis identified by screening of newborns. The American Journal of Clinical Nutrition 50(5), 1064-1071.

Solomons, N.W., Wagonfeld, J.B., Rieger, C., Jacob, R.A., Bolt, M., Horst, J.V., Rothberg, R. and Sandstead, H., 1981. Some biochemical indices of nutrition in treated cystic fibrosis patients. The American Journal of Clinical Nutrition 34(4), 462-474.

Spira, A., Gutierrez, C., Chaparro, C., Hutcheon, M.A. and Chan, C.K., 2000. Osteoporosis and lung transplantation: a prospective study. Chest 117(2), 476-481.

Stead, R.J., Houlder, S., Agnew, J., Thomas, M., Hodson, M.E., Batten, J.C. and Dandona, P., 1988. Vitamin D and parathyroid hormone and bone mineralisation in adults with cystic fibrosis. Thorax 43(3), 190-194.

Stephenson, A., Brotherwood, M., Robert, R., Atenafu, E., Corey, M. and Tullis, E., 2007. Cholecalciferol significantly increases 25-hydroxyvitamin D concentrations in adults with cystic fibrosis. The American Journal of Clinical Nutrition 85(5), 1307-1311.

Stumpf, W.E., Sar, M., Reid, F.A., Tanaka, Y. and DeLuca, H.F., 1979. Target cells for 1,25-dihydroxyvitamin D3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. Science 206(4423), 1188-1190.

Takiishi, T., Gysemans, C., Bouillon, R. and Mathieu, C., 2010. Vitamin D and diabetes. Endocrinology and Metabolism Clinics of North America 39(2), 419-446.

Tangpricha, V., Kelly, A., Stephenson, A., Maguiness, K., Enders, J., Robinson, K.A., Marshall, B.C. and Borowitz, D., 2012. An update on the screening, diagnosis, management, and treatment of vitamin D deficiency in individuals with cystic fibrosis: evidence-based recommendations from the cystic fibrosis foundation. The Journal of Clinical Endocrinology and Metabolism [Epub ahead of print] PMID:22399505.

Tejero Garcia, S., Giraldez Sanchez, M.A., Cejudo, P., Quintana Gallego, E., Dapena, J., Garcia Jimenez, R., Cano Luis, P. and Gomez de Terreros, I., 2011. Bone health, daily physical activity, and exercise tolerance in patients with cystic fibrosis. Chest 140(2), 475-481.

The UK Cystic Fibrosis Trust Bone Mineralisation Working Group (UK CF Trust), 2007. Bone mneralisation in cystic fibrosis. Report of the UK Cystic Fibrosis Trust Bone Mineralisation Working Group. Cystic Fibrosis Trust, Bromley, UK.

Thomas, M.K., Lloyd-Jones, D.M., Thadhani, R.I., Shaw, A.C., Deraska, D.J., Kitch, B.T., Vamvakas, E.C., Dick, I.M., Prince, R.L. and Finkelstein, J.S., 1998. Hypovitaminosis D in medical inpatients. New England Journal of Medicine 338(12), 777-783.

Thompson, G.N., 1987. Determinants of serum vitamin D levels in preadolescent cystic fibrosis children. Acta Paediatrica Scandinavica 76(6), 962-965.

Trang, H.M., Cole, D.E., Rubin, L.A., Pierratos, A., Siu, S. and Vieth, R., 1998. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. The American Journal of Clinical Nutrition 68(4), 854-858.

Van Belle, T.L., Gysemans, C. and Mathieu, C., 2011. Vitamin D in autoimmune, infectious and allergic diseases: a vital player? Best Practice and Research. Clinical Endocrinology and Metabolism 25(4), 617-632.

Wang, T.T., Nestel, F.P., Bourdeau, V., Nagai, Y., Wang, Q., Liao, J., Tavera-Mendoza, L., Lin, R., Hanrahan, J.W., Mader, S. and White, J.H., 2004. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. Journal of Immunology 173(5), 2909-2912.

Weisman, Y., Harell, A., Edelstein, S., David, M., Spirer, Z. and Golander, A, 1979. 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 *in vitro* synthesis by human decidua and placenta. Nature 281(5729), 317-319.

West, N.E., Lechtzin, N., Merlo, C.A., Turowski, J.B., Davis, M.E., Ramsay, M.Z., Watts, S.L., Stenner, S.P. and Boyle, M.P, 2011. Appropriate goal level for 25-hydroxyvitamin D in cystic fibrosis. Chest 140(2), 469-474.

Wills, M.R. and Savory, J, 1984. Vitamin D metabolism and chronic liver disease. Annals of Clinical and Laboratory Science 14(3), 189-197.

Wolfenden, L.L., Judd, S.E., Shah, R., Sanyal, R., Ziegler, T.R. and Tangpricha, V, 2008. Vitamin D and bone health in adults with cystic fibrosis. Clinical Endocrinology 69(3), 374-381.

Yim, S., Dhawan, P., Ragunath, C., Christakos, S. and Diamond, G., 2007. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). Journal of Cystic Fibrosis 6(6), 403-410.

Zhong, K., Chua, B.H. and Palmer, K.C, 1994. Vitamin D3 receptor expression in N-ethylnitrosourea-induced mouse pulmonary adenomas. American Journal of Respiratory Cell and Molecular Biology 11(4), 480-486.

Vitamin D and infectious disease

Key facts

- Vitamin D deficiency is a result of inadequate sun exposure and/or poor dietary intake.
- This chapter addresses the global high prevalence of vitamin D deficiency among women of childbearing age and infants and toddlers.
- Vitamin D status (nutritional and sun exposure) is assessed by measuring the transport form of vitamin D, 25-hydroxyvitamin D (25(OH)D).
- Active hormonal form is synthesized in most tissues from 25(OH)D.
- Vitamin D status is associated with immune development and lung function.
- Clinical trials are needed to establish the link between vitamin D deficiency and susceptibility to respiratory tract infections.

Summary points

- Vitamin D status of mothers and infants is poorest in darker skinned individuals, particularly those living in Northern latitudes.
- The poorest vitamin D status occurs in exclusively breast fed infants who are not supplemented with vitamin D. In contrast, formula fed infants have lower prevalence of vitamin D deficiency; however, older toddlers have limited access to vitamin D-rich foods.
- Children with Fitzpatrick skin types II to IV, in the northern and southern United States can only achieve a minimum amount of vitamin D from sun exposure during the summer months if they do not wear sunscreen at all except during beach vacations. Dark-skinned (skin types ≥ V), children fall short of achieving their needs for vitamin D from sun exposure year round.
- *In vivo* animal and *in vitro* models provide evidence that vitamin D deficiency *in utero* is associated with altered lung development and immune functions, and increasing postnatal susceptibility to respiratory infections.
- Adequate vitamin D is critical for the production of anti-microbial peptides such as cathelicidin and Beta-defensins that are key protective mechanisms in preventing respiratory infections in children under five years of age.
- Clinical trials are currently underway to determine if vitamin D supplementation reduces respiratory tract infections in infants and toddlers and during gestation in supplemented pregnant or lactating mothers.

16. Vitamin D and respiratory infections in infants and toddlers: a *nutri-shine* perspective

K.V. Balan[1], U.S. Babu[1], D.E. Godar[2] and M.S. Calvo[1]
[1]Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, 8301 Muirkirk Road, HFS-025/MOD1, Laurel, MD 20708, USA; [2]Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, US Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA; kannan.balan@fda.hhs.gov

Abstract

There is compelling evidence of a global problem of poor vitamin D status in expecting mothers and postnatal life; and even more critical, is the evidence showing the association of vitamin D deficiency with increased morbidity and mortality risks from respiratory infections. Viral and bacterial pneumonia kills more children than any other illness, accounting for 19% of all deaths in children less than five years of age worldwide; and under-nutrition, which includes vitamin D insufficiency/deficiency, has been implicated in 53% of all these deaths. Poor vitamin D status is a result of insufficient sunlight exposure and/or poor dietary intake. Greater understanding of the role of vitamin D deficiency in precipitating lung infections grew from the use of rodent models and observational and intervention studies in infants and toddlers. Vitamin D adequacy is important to maintaining the key protective mechanism of developing lungs since it mediates the synthesis of antimicrobial peptides, the lungs strongest defense against viral and bacterial pathogens. If vitamin D intervention currently under study in several clinical trials is proven successful, then implementation of new fortification practices, revised guidelines for healthy sun exposure and public health programs for vitamin D supplementation of pregnant/lactating women and their infants may be effective strategies to aide in preventing neonates and children under five from developing pneumonia. Globally, there is potential to save more than a million young lives with preventive treatment, a compelling reason why the efficacy of optimizing vitamin D mediated defense against respiratory pathogens in infants and children merits further study.

Keywords: vitamin D deficiency, infants, vitamin D supplementation, wheezing, skeletal health, respiratory infections, breast fed infants

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_16, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
CAMP	Cathelicidin antimicrobial peptide
NHANES	National Health and Nutrition Examination Survey
IOM	US Institute of Medicine
EAR	Estimated average intake
RDA	Recommended dietary allowance
RTI	Respiratory tract infection
LRTI	Lower respiratory tract infection
RSV	Respiratory syncytial virus
URTI	Upper respiratory tract infection
UVB	Ultraviolet B

16.1 Introduction

Vitamin D is a secosteroid hormone that is synthesized in humans upon solar UVB (290-315 nm) mediated conversion of endogenous skin 7-dehydrocholesterol to cholecalciferol (vitamin D3) (MacLaughlin *et al.*, 1982), and is also derived from food and dietary supplements. During the winter when adequate sunlight is limited for synthesis, there is greater dependency on dietary intake and thus the term 'vitamin' meaning essential nutrient was applied to this sunshine-derived hormone. Since vitamin D occurs naturally in a limited number of foods, fortified foods such as milk and milk products, margarines, and breakfast cereals constitute the major dietary sources of the two forms of vitamin D, cholecalciferol or ergocalciferol (vitamin D2) in the US and Canada (Calvo *et al.*, 2004). After absorption or synthesis, vitamin D2 or D3 is transported by vitamin D binding protein to the liver where the enzyme 25-hydroxylase converts it to 25(OH)D. This intermediary metabolite circulates and delivers the precursor to the active or hormonal form of vitamin D to different tissues. In this capacity, plasma 25(OH)D serves as the main status indicator for vitamin D. The mitochondrial enzyme, 1α-hydroxylase converts 25(OH)D to the active form of vitamin D, $1,25(OH)_2D$ (Holick, 2007). Renal tissues are thought to be the major source of circulating levels of $1,25(OH)_2D$, but most extra-renal tissues including the immune and airway epithelial cells constitutively or upon activation express 1α-hydroxylase and can produce $1,25(OH)_2D$ (Hansdottir and Monick, 2011).

Vitamin D has pleiotropic functions, and is involved in the regulation of approximately 1000 human genes (Tavera-Mendoza and White, 2007), including genes associated with immune responses and lung development. While its classical role in bone mineralization is well documented, the interplay of non-rachitic vitamin D status to *in utero* and post-natal health outcomes such as susceptibility to early-life respiratory infections continues to evolve. In this chapter, we review the current public health concern about vitamin D status in infants and toddlers in North America, the dietary guidelines and Federal regulations governing the level of vitamin D in foods and

supplements during pregnancy and early childhood, and examine the evidence for association between poor vitamin D status and risk of respiratory infections in infants and toddlers.

16.2 Vitamin D status and dietary guidelines during pregnancy and early childhood

Plasma 25(OH)D is considered the best biomarker for vitamin D status as it is relatively stable and reflects contributions from all sources of vitamin D (diet and endogenous synthesis). The IOM, an independent non-governmental body, recently defined adequate vitamin D status as having serum 25(OH)D concentrations greater than 50 nmol/l (or 20 ng/ml; 1 ng/ml = 2.5 nmol 25(OH)D/l) in both the general population and pregnant women, and concentrations between 30-49 nmol/l (or 12-20 ng/ml) or <30 nmol/l (or <12 ng/ml) were considered to be inadequate or deficient respectively (IOM, 2011). The IOM cutoff levels defining adequate, insufficient and deficient plasma vitamin D status are specific to bone health and do not consider other systems that are affected by circulating levels of 25(OH)D such as the immune system and respiratory health (IOM, 2011). Although serum 25(OH)D levels of at least 25 nmol/l prevents rickets, it has been proposed by some investigators that concentrations around 80 nmol/l (32 ng/ml) are optimal, since these levels lead to the greatest calcium absorption and the highest bone mass (Bischoff-Ferrari *et al.*, 2006; Dawson-Hughes, 2008) and are associated with better health outcomes involving non-skeletal tissue (Hollis, 2011).

An accurate determination of the overall dietary requirements for maintaining vitamin D adequacy is impossible because of substantial variation in skin synthesis due to season, location, age, duration of exposure, pollution, skin pigmentation and exposed area, and use of sun screen. Consequently, assuming minimal sun exposure and after thorough review of literature on skeletal health, the IOM established a RDA of 600 IU (15 μg; 1 μg=40 IU vitamin D2 or D3) vitamin D for pregnant and lactating women and toddlers. With insufficient evidence to develop an RDA in infants, an adequate intake of 400 IU (10 μg) was proposed to ensure vitamin D nutritional adequacy in this population. The IOM derives the RDA from an EAR, which was determined for vitamin D for the first time in 2011. From a public health perspective the EAR (400 IU or 10 μg/d for individuals >1 year) is used to evaluate the nutrient intake adequacy of a population at the 50th percentile (median) level of intake for all age groups (Whiting and Calvo, 2011). There appears to be global variation in recommended vitamin D intake guidelines for pregnant women, infants and toddlers (Table 16.1). This variation could be a result of differences due to season, demographics of light versus dark skinned individuals, national supplementation and fortification policies, and more importantly skepticism associated with non-skeletal health benefits from basic science experiments and observational studies as compared to randomized clinical trials.

16.2.1 Maternal status of vitamin D levels

Maternal vitamin D deficiency during pregnancy is global and widely prevalent, and can be attributed to inadequate vitamin D intake and restricted sunlight exposure during winter months

Table 16.1. Examples of global recommendations for dietary vitamin D intake (IU/d) (FAO/WHO, 2001; Holick *et al.*, 2011; IOM, 1997, 2011; Vidailhet *et al.*, 2012).

Year	Country	3 months[1]	9 months[1]	5 years	Pregnancy[2]
1991	United Kingdom	340	280	–[3]	–[3]
1996	Italy	400	700	200	200
1997	IOM (United States/Canada)	200	200	200	200
2001	France	800-1000	800-1000	200	200
2001	World Health Organization (WHO)	200	200	200	200
2004	Nordic countries	400	400	300	300
2004	Germany/Austria/Switzerland	400	400	200	200
2005	Australia and New Zealand	200	200	200	200
2011	IOM (United States/Canada)	400	400	600	600
2011	The Endocrine Society – United States	400-1000	400-1000	600-1000	1,500-2,000
2012	France	1000-1,200	1000-1,200	2 doses[4] of 80,000- or 100,000-IU in winter – Nov & Feb	1 dose[4] of 80,000- or 100,000-IU @ start of 3rd trimester

[1] Breastfed infants.

[2] Below 50 yrs of age.

[3] 400 IU/d in cases of insufficient UVB exposure.

[4] Bolus dose.

compared to summer months, northern latitudes, and higher melanin levels in dark-skinned or veiled women. Based on a National Human Activity Pattern Survey it has been estimated from the outdoor-sunlight data that US women including those of child-bearing age (22-40 years) get lower annual UVB doses than males because they spend less time outdoors (Godar, 2001; Godar *et al.*, 2001). In the US, 29% of African-American pregnant women and 5% of Caucasian pregnant women residing in the northern latitudes are vitamin D deficient (defined as serum 25(OH)D less than 37.5 nmol/l); whereas 54% of African-American participants and 47% of Caucasian participants are vitamin D insufficient (defined as serum 25(OH)D 37.5-80 nmol/l) (Bodnar *et al.*, 2007). Moreover, 18% of pregnant women in the United Kingdom, 25% in the United Arab Emirates, 80% in Iran, 42% in northern India, 61% in New Zealand and 60-84% of pregnant non-Western women in the Netherlands have been shown to have serum 25(OH)D concentrations <25 nmol/l (Dawodu and Wagner, 2007). Thus, there is concern that *in utero* vitamin D deficits could lead to developmental re-programming of body functions and long-term morbidity in infants, children and adults.

Cumulative intake data from the nationally representative NHANES survey conducted from 2007-2008 show that US women of childbearing age have significantly lower (P<0.01) vitamin D intakes than their age-matched male counterparts (Figure 16.1) (USDA, 2010). A comprehensive assessment of 25(OH)D serum data from participants in NHANES 2001-2006, one year of age and over, after adjusting for age and season, showed that males are less likely to be at risk of deficiency (<30 nmol/l) than females (Figure 16.2) and the prevalence of those at risk of inadequacy (30-49 nmol/l) did not differ by sex. However, in the 2001-2006 NHANES cohort, among women of childbearing age, those who were pregnant or lactating were less likely to be at risk of deficiency compared to women who were not pregnant or lactating (Figure 16.2) and no differences in prevalence of vitamin D inadequacy were observed between the two groups (Looker *et al.*, 2011). These prevalence data reflect 24 hr recall levels of vitamin D intake, which suggests that pregnant and lactating women in general in the US are in compliance with the current dietary guidelines for maintaining vitamin D status or have adequate sun exposure. A rigorous analysis of current NHANES data is needed to determine the association of vitamin D status with sun exposure and total 'usual' dietary intake for individual race/ethnicities, since smaller cross-sectional studies have shown association of maternal deficiency, race (African-American), winter birth, and a BMI of ≥35 as risk factors for newborn vitamin D deficiency.

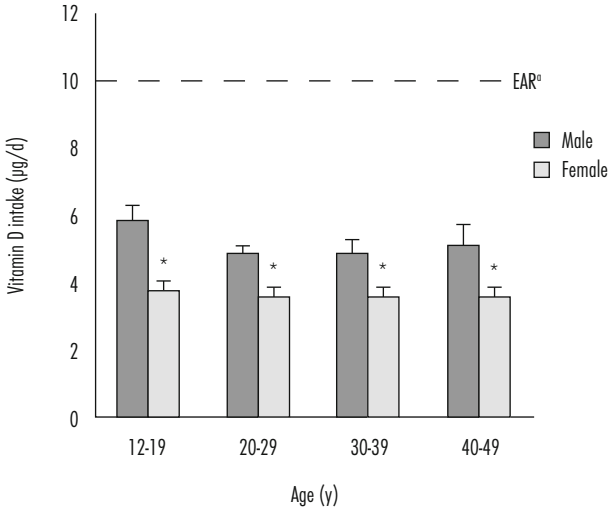

Figure 16.1. Vitamin D intake in the United States (NHANES, 2007-2008). Mean vitamin D intake (±SE) from food per individual, by gender and age of participants in the 2007-2008 NHANES survey of US population.
[a] Estimated average requirement (EAR) for vitamin D intake established in 2011 (IOM, 2011). The differences in vitamin D intake between males and females were significant across all age groups (P<0.01) (USDA, 2010).

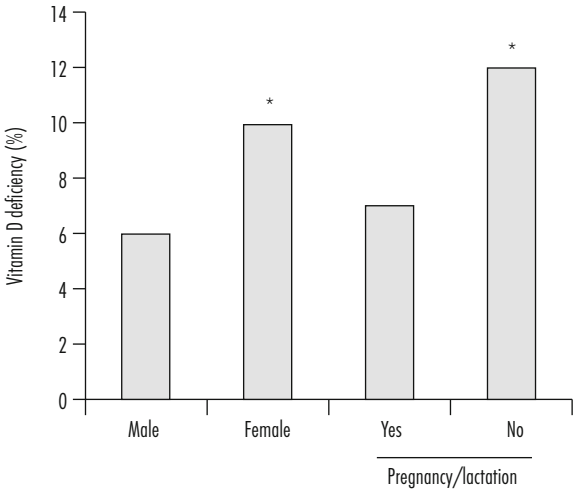

Figure 16.2. Prevalence of vitamin D deficiency in the United States (NHANES, 2001-2006).
Age- and season-adjusted prevalence of risk for vitamin D deficiency (<30 nmol/l) among men and women, and among women of childbearing age who were pregnant or lactating (Yes) or not (No) among participants of the 2001-2006 NHANES survey. * Indicates significant difference between males and females and between women's pregnancy/lactation status at $P<0.05$. Figure modified from Looker *et al.* (2011).

16.2.2 Vitamin D status in infants

During pregnancy the fetus is wholly dependent on the mother for vitamin D, and 25(OH)D readily crosses the placenta such that cord blood 25(OH)D levels are between 80% and 100% of maternal concentrations (Fleischman *et al.*, 1980; Gertner *et al.*, 1980; Hillman and Haddad, 1974). There is a strong correlation between maternal and newborn 25(OH)D circulating levels as the half-life of 25(OH)D is approximately 2 to 3 weeks, and a low maternal status often results in the infant being deficient in vitamin D during the first weeks post-partum (Hollis and Wagner, 2004a; Yu *et al.*, 2009). Because vitamin D secretion in breast milk is limited (Kovacs, 2008), lactating women require robust serum 25(OH)D levels to support vitamin D status in nursing infants (Hollis and Wagner, 2004b). The World Health Organization recommends supplementation with 400-IU daily vitamin D to all pregnant women; however, because of lack of awareness and poor compliance, 46% of newborns in industrialized countries are born with insufficient serum levels of 25(OH)D (Belderbos *et al.*, 2011).

More recently Balk and the Council on Environmental Health and Section on Dermatology recommended that children younger than 1-year of age avoid direct sunlight and also use sunscreen (Balk, 2011). Furthermore, for reasons that are not entirely clear, data suggest that the relationship between vitamin D intake and serum 25(OH)D levels is non-linear (Hypponen *et al.*, 2009), and current guideline levels for vitamin D supplementation (200-600 IU/d) are unlikely to achieve optimal serum 25(OH)D levels (IOM, 2011). In a study of 40 mother-infant pairs, 76% of mothers and 81% of newborns had a 25(OH)D level below 50 nmol/l at the time

of birth, despite the fact that during pregnancy the mothers ingested about 600 IU/d of vitamin D from a prenatal supplement and consumed two glasses of milk (Lee *et al.*, 2007). Thus, it is understandable that infants who are fed only human breast milk are prone to developing vitamin D deficiency leading to a Federal mandate both in the United States and Canada to fortify infant formula with vitamin D at 40-100 IU/kcal and 40-80 IU/kcal, respectively. The effectiveness of infant formula fortification was evident in a cross-sectional study of 247 healthy infants in a primary care setting in Boston as breastfeeding without supplementation markedly increased the odds of vitamin D deficiency compared with infants who were exclusively formula (bottle)-fed (Gordon *et al.*, 2008). Interestingly, although maternal supplementation of 200-IU/d vitamin D was available to all nursing mothers, there may be barriers to obtaining the supplement or lack of awareness of the need to supplement, which resulted in poor compliance. Thus, with emerging data on insufficiency in vitamin D levels with the IOM 1997 recommendation of 200 IU/d, the revised recommendation is now set at 600 IU/d for infants in the United States and Canada (IOM, 2011).

16.2.3 Vitamin D status in toddlers

Most American children may not be going outside enough to meet their vitamin D3 needs from sun exposure (Godar *et al.*, 2012). Recent estimates from everyday outdoor exposure suggest that children (≤5 years) in the northern (45°N) (Figure 16.3a) and southern (35°N) (Figure 16.3b) United States with Fitzpatrick Type II skin (Caucasian) (Fitzpatrick, 1988) can have adequate skin synthesis of vitamin D3 during the summer and children living in the south during the spring as well but only if they do not wear sunscreen at all except during beach vacations. Children with Fitzpatrick Type III and IV skin (olive tone-Hispanic/Asian or brown tone-Indian, respectively)

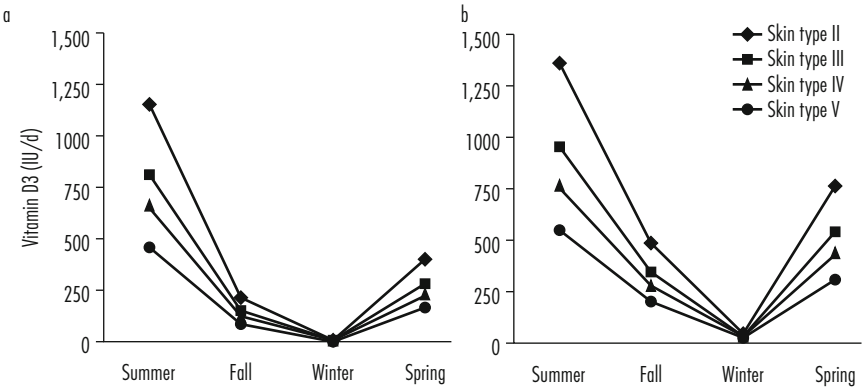

Figure 16.3. Estimated cutaneous synthesis of vitamin D3 in toddlers according to skin type and season. Average estimated vitamin D3 produced in toddlers (≤5 years of age) from everyday outdoor UV exposures without the use of sunscreens in (a) the northern (45°N) or (b) southern (35°N) United States according to skin type and season. Skin types represent Fitzpatrick skin types II (Caucasian), III (olive skin tone, Hispanic or Asian), IV (brown skin tone, e.g. Indian) and V (light to moderate-skinned African American) (Fitzpatrick, 1988).

can only synthesize the suggested minimum daily recommendation during the summer months, while those with the darkest pigmentation (skin type V and higher, usually African-Americans) never make the suggested minimum amount of vitamin D3 from sun exposure (Godar *et al.*, 2012). These vitamin D3 estimates from solar exposures assume certain skin types, liberal clothing scenarios during each season and that sunscreens are not worn except during beach vacations. These could be overestimates for infants and toddlers since mothers tend to be overly protective of their children to avoid skin cancer later in life or could be a result of children spending more time indoors watching television. Despite the fact that younger children are spending more time indoor, children in the younger age group (≤5 years) have more cutaneous vitamin D synthesis than those in the older age groups (Godar, 2001).

With children falling short of the daily needed levels of vitamin D3 from sunshine exposure especially in the winter months, fortified foods are key to maintain adequate vitamin D status in toddlers, and increasingly, the manufacturers of foods targeted to toddlers are recognizing the need to fortify with vitamin D. Levels of vitamin D fortification range from 1 μg (40 IU) per regulatory serving for ready-to-eat cereals to 2.5 μg for fluid milk servings (Calvo and Whiting, 2013). It is evident from reviewing the vitamin D content of the 'baby foods' listed in the recent USDA database, Standard Release-24 for nutrient composition of foods that there is a need for newer fortified baby foods. A review of 223 foods showed vitamin D contents ranged from 0 to 0.8 μg vitamin D per 100 g, and approximately 90% had no listed vitamin D content. With the availability of limited fortified baby foods, in a population study of 750 healthy children aged 6-36 months, the mean overall vitamin D intake from food plus supplements was 290±124 IU/d in 6-12 month age group and 240±128 IU/d in 1-3 years age group (Figure 16.4), and group corresponding serum 25(OH)D levels decreased with age. A quite concerning trend in this cohort

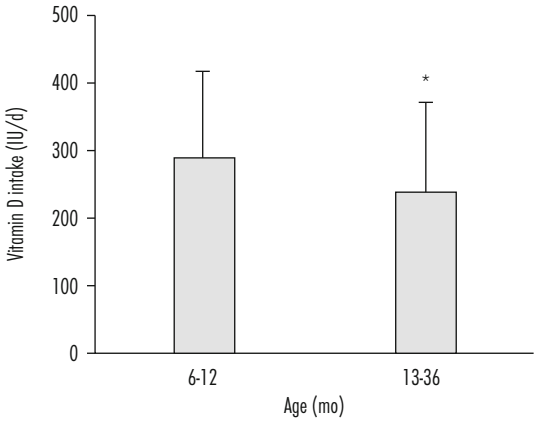

Figure 16.4. Effect of lack of vitamin D-fortified foods on the daily dietary intake in a cohort of healthy infants and toddlers. Vitamin D daily dietary intake (±SD) in 6-12 months (n=131) and 13-36 months old (n=645) healthy children. Significant age differences in vitamin D intake, $P<0.001$. Figure drawn from Carpenter *et al.* (2012).

was that children discontinued breastfeeding by 5 months of age and most children (84%) were no longer formula-fed at 5 months (Carpenter *et al.*, 2012). Thus, with the IOM recommended adequate intake of 400 IU/d for infants and 600 IU/d for toddlers approximately 82% of infants and 2% of toddlers met the age-specific dietary intake recommendation. These findings suggest that vitamin D-fortified infant formulas provide a positive effect on vitamin D levels and similar supplementary practices are likely to be effective in increasing 25(OH)D status in young children (Carpenter *et al.*, 2012). The mean intake data for toddlers (2-5 years age) from the NHANES 2007-2008 cohort shows a significant difference in vitamin D intake among the racial and ethnic groups with Non-Hispanic blacks consuming less than Non-Hispanic whites, who consume less than Mexican Americans (Figure 16.5) (USDA, 2010). Correspondingly, the prevalence of vitamin D insufficiency and deficiency in children (1-5 years) during the 2001-2004 NHANES was highest among Non-Hispanic blacks followed by Mexican Americans and Non-Hispanic whites (Figure 16.6) (Kumar *et al.*, 2009).

There is clear evidence for global and widespread vitamin D deficiency or insufficiency due to various risk factors including lack of sunlight exposure, seasonal variation, suboptimal dietary intake, and dark skin pigmentation. More importantly, we have shown the greatest barriers to adequate vitamin D from sun exposure or diet occur in individuals with the poorest vitamin D status. How this widespread deficiency relates to the risk of respiratory tract infections in infants and toddlers is less clear and merits further study.

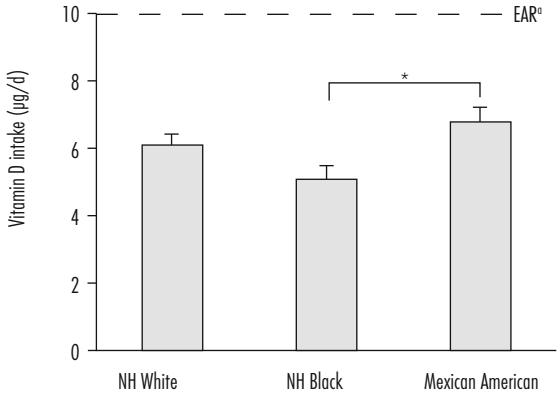

Figure 16.5. Vitamin D intake among toddlers in the United States, NHANES, 2007-2008. Mean intake (±SE) of vitamin D (D2 + D3) from NHANES 2007-2008 for toddlers 2-5 years, presented by race and ethnic group. [a] EAR for vitamin D intake established in 2011 (IOM, 2011). NH: Non-Hispanic. * Indicates significant difference in vitamin D intake between NH Black and Mexican Americans, $P<0.01$. Figure drawn with data from USDA (2010).

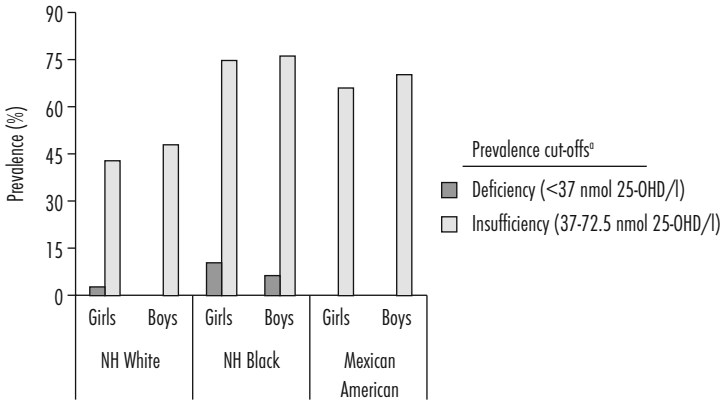

Figure 16.6. Prevalence of vitamin D deficiency among toddlers in the United States, NHANES, 2001-2004. Prevalence of vitamin D insufficiency (defined as serum 25(OH)D levels 37-72.5 nmol/l) and deficiency (defined as serum 25(OH)D levels <37 nmol/l) among toddlers and children (1-6 years of age) in NHANES 2001-2004. NH: Non-Hispanic. [a] Values differ from IOM (2011). Figure redrawn from Kumar *et al.* (2009).

16.3 Fetal and postnatal outcomes associated with vitamin D status

The fetal origins hypothesis, first articulated by David Barker, postulates that *in utero* epigenetic fetal programming, as a result of environmental events during pregnancy, induces specific genes and genomic pathways that control fetal development and subsequent disease risk (Barker *et al.*, 2002). The biological effects of active vitamin D are achieved through the regulation of gene expression in a cell and tissue specific manner. Briefly, active vitamin D binds to the vitamin D nuclear receptor, and initiates dimerization with the retinoic X receptor. This active complex binds to the nuclear vitamin D responsive elements within the promoter regions of vitamin D-specific responsive genes and initiates gene expression (MacDonald *et al.*, 1993). The developmental periods of *in utero* and infancy represent critical periods of dynamic development and maturation of key processes. Therefore, in the following sections, we focus on the role of vitamin D in modulating lung structure and innate immune functions, a primer for susceptibility to viral respiratory tract infections during infancy and early childhood (Figure 16.7).

16.3.1 Lung development

Lung development occurs predominantly before birth (Stick, 2000) with extensive interactions between epithelial and mesenchymal tissue beginning by the fourth week of gestation and continuing for years after birth, and factors that impair fetal and early childhood lung development have the potential to exert major effects on lung function during childhood and adulthood. Despite differences in the epithelial growth and differentiation during lung development in rodents and humans, rodent models are widely used for lung developmental studies. Rodent models act as a bridge between studies in the laboratory and studies in humans, and have been used to study pulmonary infections (Balan *et al.*, 2011). In rodents, it has been demonstrated that vitamin

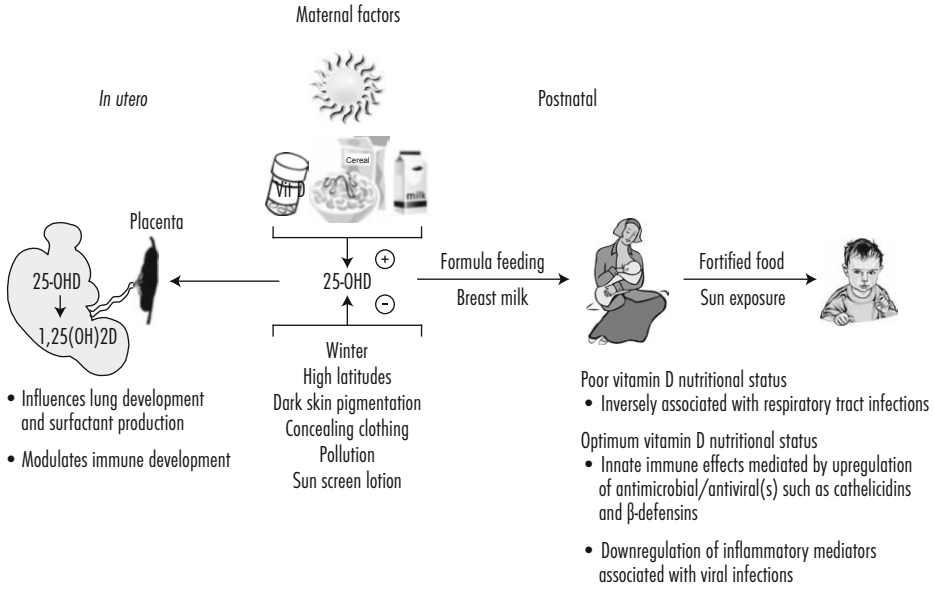

Figure 16.7. Role of nutrition and sunshine in maintaining vitamin D adequacy and reducing incidence of respiratory tract infections in postnatal life.

D modulates key alveolar epithelial-mesenchymal interactions, such as type II pneumocyte and lipofibroblast proliferation and differentiation which are critical for alveolar development and septal thinning during perinatal pulmonary maturation (Sakurai *et al.*, 2009). Vitamin D-deficient rodent models provide mechanistic evidence for a causal link between vitamin D deficiency and deficits in lung function and altered lung structure. Specifically, offspring of vitamin D-deficient mice showed no effect on the overall somatic growth, had marginal reduced numbers of alveoli, and exhibited physiologically significant decreases in lung volume and altered lung mechanics when compared with offspring of vitamin D-replete mice (Zosky *et al.*, 2011). This mouse study complements data showing decreased lung compliance in 50-d-old rats born to vitamin D-deficient mothers (Gaultier *et al.*, 1984), confirming the relationship between vitamin D deficiency and lung function. In addition, as vitamin D deficiency has the potential for premature births in humans (Dawodu and Nath, 2011), studies have shown that prematurity leads to diminished lung function and may predispose premature infants to severe viral lower respiratory tract infections in infancy (Drysdale *et al.*, 2011).

Active vitamin D synthesis has been shown in rodent fetal lung fibroblasts, whereas adjacent alveolar type II pneumocytes express vitamin D receptors and respond to the hormone by undergoing differentiation and maturation resulting in decreased cell glycogen content, and increased surfactant synthesis and secretion (Nguyen *et al.*, 1996, 2004). Surfactant proteins were initially identified as a lipoprotein complex that reduced surface tension at the air-liquid interface of the lung, but recent studies have identified surfactant proteins as components of the lung innate and adaptive immune system with novel roles in the direct killing of inhaled microorganisms and

viruses, and control of pulmonary inflammation (Wright, 2005). The vitamin D-mediated rodent type II pneumocyte maturation data looks intriguing but the relevance to humans is uncertain. Regulation of surfactant protein gene expression in human fetal type II pneumocytes by active vitamin D is not coordinated as it is in rodents, but vitamin D receptor immuno-staining is observed in human fetal fibroblasts and type II pneumocytes (Phokela *et al.*, 2005; Stio *et al.*, 1997).

Thus, there is evidence for *in utero* vitamin D deficiency and association with altered epithelial-mesenchymal maturation, lung mechanics, and immunoregulatory surfactant production. While the association between surfactants in providing innate immunity against respiratory viral infections is conceivable, the role of altered pulmonary function with vitamin D deficiency could also indicate an association with asthma, a chronic inflammatory condition that is described in detail in a separate chapter.

16.3.2 Vitamin D and innate immune protection

The fetus and neonate face a complex set of immunologic demands, and vitamin D has been shown to have an important role in the innate immune system, which helps to prevent infection without the need for immunologic memory from previous exposure to the pathogen (Adams and Hewison, 2008). Innate immunity includes the production of antimicrobial peptides such as Beta-defensins and CAMP by epithelial cells and circulating leukocytes, which are capable of killing a variety of respiratory pathogens including viruses, bacteria, and fungi (Hiemstra, 2007). In early gestation, human decidual cells have been shown to synthesize active vitamin D, which may exert autocrine or paracrine effects on the developing fetal immune system. Vitamin D mediates expression of mRNA for CAMP in decidual cells (Evans *et al.*, 2006), and has been linked to intrauterine immunity (Singh *et al.*, 2005). Human monocytes in an *in vitro* model, when supplemented with 25(OH)D-deficient cord blood plasma showed a significant decrease in CAMP expression, thus correlating with increased susceptibility to newborn infections (Walker *et al.*, 2011).

In postnatal life, respiratory epithelial cells provide a barrier between the outside environment and internal parenchyma, and are primary targets of respiratory pathogens. The respiratory epithelial cells constitutively activate vitamin D, and are capable of creating a microenvironment that has high levels of active form of the vitamin, resulting in the activation of downstream genes such as those for CAMP. Moreover, viral RNA increases the expression of 1α-hydroxylase, leading to increased activation of vitamin D and further increases in CAMP mRNA (Hansdottir *et al.*, 2008). Hansdottir and colleagues (2010) have shown in an *in vitro* human tracheo-bronchial epithelial cell model for respiratory syncytial virus infection, that vitamin D attenuates inflammatory cytokine and chemokine response, while maintaining the antiviral activity. This local vitamin D-mediated anti-viral, anti-inflammatory immune response could result in decreased disease severity and morbidity from this common infection (Hansdottir *et al.*, 2010). Thus, vitamin insufficiency and notably, a seasonal decrease of vitamin D-dependent epithelial and leukocyte innate host defense could contribute to increased susceptibility to respiratory infections during

winter. A prospective descriptive study of outcomes associated with vitamin D deficiency and pneumonia reported 25(OH)D deficiency associated with increased mortality but not associated with levels of cathelicidin or Beta-defensin (Leow *et al.*, 2011). Clinical trials are underway to assess CAMP expression as a biomarker for fetal-neonatal immune function following antenatal vitamin D supplementation (ClinicalTrials.gov identifier: NCT01126528) and in a separate trial for Intensive Care Unit-associated lung failure, CAMP and Beta-defensin concentrations will be evaluated as a secondary outcome following high-dose vitamin D regimen (ClinicalTrials.gov identifier: NCT01372995).

While we limit our review to the role of innate immune antiviral functions to respiratory infections, vitamin D status has been associated with other prenatal and postnatal immunomodulatory effects, such as promoting peripheral tolerance by rendering antigen presenting dendritic cells tolerogenic and development of T-regulatory cells. Dendritic and T-regulatory cells serve as pivotal links between innate and adaptive immunity, and play a key role in the protection against the inflammatory sequela of airway infections and in the protection against induction and expression of atopic disease (Chambers and Hawrylowicz, 2011; Holt *et al.*, 2008).

16.4 Evidence for vitamin D status and risk of respiratory infections & childhood wheezing

There is an association of wintertime peaks in respiratory infections especially in the higher latitudes to vitamin D status as the cutaneous synthesis of vitamin D is naturally blunted during that time of the year (Cannell *et al.*, 2006). Respiratory tract infections in the neonatal and pediatric populations are normally viral in origin with accompanying wheezing, pneumonia or bronchiolitis. The infections can be classified into URTIs and LRTIs with the URTI a primary site of contact for inhaled agents, and LRTIs as infections of the intra-thoracic airways and/or lung parenchyma with severe cases leading to bronchiolitis and pneumonia. The main etiological agents include human rhinovirus, RSV, human coronavirus, adenovirus, parainfluenza virus and influenza virus. Cases of bronchiolitis associated with human rhinovirus (46-49%), RSV (11-27%), and parainfluenza viruses (5-13%) account for most of the outpatient sampling in infants with URTIs and LRTIs (Camargo *et al.*, 2011a). Viral infections including rhinovirus and RSV could lead to bronchiolitis and early episodic wheezing in infants (Gern and Busse, 2002), symptoms which are commonly associated with the likelihood of developing reactive airway disease or asthma in early childhood (Asher *et al.*, 2006; Litonjua, 2012; Wu *et al.*, 2008). Large cohort studies, however have demonstrated that many children who wheeze in early childhood during acute respiratory infections do not go on to develop asthma (Camargo *et al.*, 2011b; Martinez *et al.*, 1995; Stein and Martinez, 2004). In addition, accumulating evidence implicates a background of atopy as a leading cause of asthma associated with airway inflammation from respiratory infections (Holt *et al.*, 2012). Nevertheless, asthma is a heterogeneous disease and since clinical diagnosis of asthma in children remains a challenge, epidemiological studies of children often focus on childhood wheezing; and in this chapter, we present selected epidemiological studies

on vitamin D deficiency in children and associated respiratory infections and wheezing but not related to asthmatic conditions.

16.4.1 Observational prospective serum 25(OH)D birth cohort studies

Circulating 25(OH)D level provides a distinct advantage to assess vitamin D status than self-reported dietary intake, and cord-blood 25(OH)D concentration is strongly associated with maternal concentration during pregnancy. In a birth cohort study of 922 children cord-blood levels of 25(OH)D had inverse associations with the risk of respiratory infection by three months of age (OR: 1.00 for ≥75 nmol/l, 1.39 for 25-74 nmol/l, and 2.16 for <25 nmol/l). Likewise, cord-blood 25(OH)D levels were inversely associated with risk of wheezing by 15 months, 3 years, and 5 years of age (all $P<0.05$) and no association to incident asthma by the age of 5 years. Additional adjustment for potential confounders including seasons of birth did not materially change these results (Camargo *et al.*, 2011b). A recent birth cohort study of 156 healthy neonates showed an association of vitamin D deficiency with increased risk of RSV LRTIs in the first year of life. Neonates born with 25(OH)D concentrations <50 nmol/l had a sixfold (95% CI=1.6-24.9; $P=0.01$) increased risk of RSV LRTI in the first year of life compared with those with 25(OH)D concentrations ≥75 nmol/l (Belderbos *et al.*, 2011). Similarly, in a population based mother-child cohort study there was a trend of independent association between higher levels of maternal circulating 25(OH)D in pregnancy and decreased odds of LRTIs in offspring (for cohort- and season-specific quartile Q4 vs. Q1, OR: 0.67 (95% CI=0.50-0.90); test for trend, $P=0.016$). No association was found between 25(OH)D levels in pregnancy and risk of wheezing at age 1 year or 4 years, or asthma at age 4-6 years (Morales *et al.*, 2012).

16.4.2 Case-control studies

Nutritional rickets due in part to vitamin D deficiency is a major health problem in developing countries. It is associated with respiratory muscle weakness and increased risk of respiratory infections. The first association of sub-clinical nutritional rickets to respiratory infections was observed in a case-control trial among Indian children (3-12 years) with multiple episodes of respiratory infections. Administration of oral vitamin D, 60,000 IU/wk and 650 mg of calcium/d for 6 weeks decreased the incidence of respiratory infections in the test population (Rehman, 1994). Similarly in a case-control study, 13-fold higher incidence of nutritional rickets was observed among toddlers (<5 years) with pneumonia than among controls (OR=13.37; $P<0.001$) (Muhe *et al.*, 1997). More recently, significant associations have been identified with non-rachitic vitamin D levels and respiratory tract infections suggesting that vitamin D insufficiency is enough to trigger respiratory infection rather than a secondary manifestation of acute deficiency typically leading to nutritional rickets.

In a hospital-based case-control study of non-rachitic Indian children age 2-60 months, serum 25(OH)D levels of <22 nmol/l had more than 10-fold higher odds of acquiring severe acute LRTIs (OR: 0.09; $P<0.001$) (Wayse *et al.*, 2004), and in a similar study design in rural Bangladesh, 25(OH)D levels in children (1-18 months) hospitalized with acute lower respiratory infections

(ALRIs) were significantly lower (29.1 nmol/l) than case matched controls (39.1 nmol/l). The unadjusted odds ratio of ALRIs was halved for each 10 nmol/l increase in 25(OH)D (OR=0.53; 95% CI=0.3, 0.96) (Roth *et al.*, 2010). Furthermore, in a neonatal case-control study of ALRIs in Turkey, mean serum level of 25(OH)D was 22.7±22.2 nmol/l compared to age-matched control levels, 40.6±33.6 nmol/l (P=0.011) and corresponding 25(OH)D levels in mothers of the study group were lower than those in the mothers of the control group (33.4±42 nmol/l and 57±42.3 nmol/l respectively; P=0.012). These findings suggest that newborns with subclinical vitamin D deficiency may have an increased risk of suffering from acute lower respiratory infection and the strong positive correlation between newborns and mothers 25(OH)D concentrations shows that adequate vitamin D supplementation of mothers should be emphasized during pregnancy (Karatekin *et al.*, 2009).

In contrast, two case-control studies in Canadian children did not reveal an association between vitamin D deficiency and respiratory infections as virtually all children consumed vitamin D fortified infant formula or supplements (Roth *et al.*, 2009). While no difference was observed in vitamin D levels between the entire acute lower respiratory infection group and control group, approximately 50% of the patients admitted to the pediatric intensive care unit were vitamin D deficient (<50 nmol/l) compared to only 20% on the general medical floor (OR: 8.23; 95% CI: 1.4, 48.0; P=0.02) suggesting that low levels of vitamin D predispose to greater acute LRTI severity (McNally *et al.*, 2009).

16.4.3 Interventional trials

Published data from randomized control clinical trials to evaluate the effects of vitamin D on reducing RTIs in children are limited. Despite mixed results because of study design, lack of measurement of 25(OH)D serum levels and poor compliance, four studies have shown potential for vitamin D intervention to help control respiratory tract infections in the adult population (Aloia and Li-Ng, 2007; Avenell *et al.*, 2007; Laaksi *et al.*, 2007; Li-Ng *et al.*, 2009).

A supplementation of vitamin D (1,200 IU/d) for four winter months among 334 schoolchildren in Japan showed a reduction in influenza A (10.8%) children in the vitamin D group compared with (18.6%) children in the placebo group [relative risk (RR), 0.58; 95% CI: 0.34, 0.99; P=0.04]. In a sub-group analysis significant reductions of influenza A were more prominent in children taking additional vitamin D supplements (RR 0.36; 95% CI: 0.17-0.78). The study, however, lacked measurements of serum 25(OH)D and serum antibody concentrations to influenza A (Urashima *et al.*, 2010). A current search of the ClinicalTrials.gov database lists the Vitamin D Outcomes and Interventions In Toddlers (DO IT Trial: NCT01419262, Primary Outcome: Respiratory Infection) and Maternal Vitamin D Supplementation to Prevent Childhood Asthma (VDAART Trial: NCT00920621, Secondary Outcome: LRTIs in the first 3 years of life) as ongoing trials to evaluate the effects of vitamin D on reducing RTIs in infants and toddlers. The gestational supplementation of 4,000 IU/d in the VDAART trial is noted as a recent trial on evaluating clinical safety and effectiveness of vitamin D supplementation during pregnancy concluded that

supplementation of 4,000 IU/d is safe and most effective in achieving sufficiency in all women and their neonates regardless of race (Hollis *et al.*, 2011).

16.5 Summary and conclusion

We have presented what we believe is compelling evidence of a global problem of poor vitamin D status experienced *in utero* and perinatally; and even more critical, is the evidence showing the association of vitamin D deficiency during this dynamic period of development with increased morbidity and mortality risks from respiratory infections. Worldwide recognition of this problem is growing and this awareness is stimulating changes in dietary guidelines (Table 16.1), as well as advice on safe sun exposure (Godar *et al.*, 2012) and strategies for national fortification policies (Babu and Calvo, 2010). Viral and bacterial pneumonia kills more children than any other illness, accounting for 19 per cent of all under five deaths worldwide (UNICEF, 2006). According to the 2006 UNICEF/WHO report, an estimated 26% of neonatal deaths are caused by severe infections during the neonatal period, the majority of which are caused by pneumonia/sepsis. Undernutriton, which includes vitamin D insufficiency/deficiency, has been implicated in 53% of all deaths among children under five (UNICEF, 2006). If vitamin D intervention currently under study in the clinical trials described in this chapter is proven successful, then implementation of new fortification practices, revised guidelines for healthy sun exposure and public health programs for vitamin D supplementation of pregnant/lactating women and their infants may be effective strategies to aid in preventing neonates and children under five from developing respiratory infections. Globally, there is potential to save more than a million young lives with preventive treatment, a compelling reason why the efficacy of optimizing vitamin D-mediated defense against respiratory pathogens in infants and children merits further study.

Disclaimer

The findings and conclusions presented in this review are those of the authors and do not necessarily represent the views, opinions or policies of the US Food and Drug Administration.

References

Adams, J.S. and Hewison, M., 2008. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nature Clinical Practice: Endocrinology & Metabolism 4, 80-90.

Aloia, J.F. and Li-Ng, M., 2007. Re: epidemic influenza and vitamin D. Epidemiology and Infection 135, 1095-1096; author reply 1097-1098.

Anonymous, 2011. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. The National Academies Press (US), Washington (DC), USA, 1115 pp.

Asher, M.I., Montefort, S., Bjorksten, B., Lai, C.K., Strachan, D.P., Weiland, S.K. and Williams, H., 2006. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 368, 733-743.

Avenell, A., Cook, J.A., Maclennan, G.S. and Macpherson, G.C., 2007. Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). Age and Ageing 36, 574-577.

Babu, U.S. and Calvo, M.S., 2010. Modern India and the vitamin D dilemma: evidence for the need of a national food fortification program. Molecular Nutrition & Food Research 54, 1134-1147.

Balan, K.V., Kc, P., Mayer, C.A., Wilson, C.G., Belkadi, A. and Martin, R.J., 2011. Intrapulmonary lipopolysaccharide exposure upregulates cytokine expression in the neonatal brainstem. Acta Paediatrica 101(5), 466-471.

Balk, S.J., 2011. Ultraviolet radiation: a hazard to children and adolescents. Pediatrics 127, e791-817.

Barker, D.J., Eriksson, J.G., Forsen, T. and Osmond, C., 2002. Fetal origins of adult disease: strength of effects and biological basis. International Journal of Epidemiology 31, 1235-1239.

Belderbos, M.E., Houben, M.L., Wilbrink, B., Lentjes, E., Bloemen, E.M., Kimpen, J.L., Rovers, M. and Bont, L., 2011. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics 127, e1513-1520.

Bischoff-Ferrari, H.A., Giovannucci, E., Willett, W.C., Dietrich, T. and Dawson-Hughes, B., 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. American Journal of Clinical Nutrition 84, 18-28.

Bodnar, L.M., Simhan, H.N., Powers, R.W., Frank, M.P., Cooperstein, E. and Roberts, J.M., 2007. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. Journal of Nutrition 137, 447-452.

Calvo, M.S. and Whiting, S.J., 2013. Vitamin D fortification in North America: current status and future considerations. In: V. Preedy (ed.), Handbook of food fortification and health: from concepts to public health applications. King's College London, London, UK (in press).

Calvo, M.S., Whiting, S.J. and Barton, C.N., 2004. Vitamin D fortification in the United States and Canada: current status and data needs. American Journal of Clinical Nutrition 80, 1710S-1716S.

Camargo Jr., C.A., Ginde, A.A. and Mansbach, J.M., 2011a. Vitamin D, Childhood Wheezing, Asthma, and Chronic Obstructive Pulmonary Disease. In: D. Feldman, W.J. Pike and J.S. Adams (eds.), Vitamin D. Academic Press, Oxford, UK, pp. 1999–2021.

Camargo Jr., C.A., Ingham, T., Wickens, K., Thadhani, R., Silvers, K.M., Epton, M.J., Town, G.I., Pattemore, P.K., Espinola, J.A. and Crane, J., 2011b. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. Pediatrics 127, e180-187.

Cannell, J.J., Vieth, R., Umhau, J.C., Holick, M.F., Grant, W.B., Madronich, S., Garland, C.F. and Giovannucci, E., 2006. Epidemic influenza and vitamin D. Epidemiology and Infection 134, 1129-1140.

Carpenter, T.O., Herreros, F., Zhang, J.H., Ellis, B.K., Simpson, C., Torrealba-Fox, E., Kim, G.J., Savoye, M., Held, N.A. and Cole, D.E., 2012. Demographic, dietary, and biochemical determinants of vitamin D status in inner-city children. American Journal of Clinical Nutrition 95, 137-146.

Chambers, E.S. and Hawrylowicz, C.M., 2011. The impact of vitamin D on regulatory T cells. Current Allergy and Asthma Reports 11, 29-36.

Dawodu, A. and Nath, R., 2011. High prevalence of moderately severe vitamin D deficiency in preterm infants. Pediatrics International 53, 207-210.

Dawodu, A. and Wagner, C.L., 2007. Mother-child vitamin D deficiency: an international perspective. Archives of Disease in Childhood 92, 737-740.

Dawson-Hughes, B., 2008. Serum 25-hydroxyvitamin D and functional outcomes in the elderly. American Journal of Clinical Nutrition 88, 537S-540S.

Drysdale, S.B., Wilson, T., Alcazar, M., Broughton, S., Zuckerman, M., Smith, M., Rafferty, G.F., Johnston, S.L. and Greenough, A., 2011. Lung function prior to viral lower respiratory tract infections in prematurely born infants. Thorax 66, 468-473.

Evans, K.N., Nguyen, L., Chan, J., Innes, B.A., Bulmer, J.N., Kilby, M.D. and Hewison, M., 2006. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. Biology of Reproduction 75, 816-822.

Fitzpatrick, T.B., 1988. The validity and practicality of sun-reactive skin types I through VI. Archives of Dermatology 124, 869-871.

Fleischman, A.R., Rosen, J.F., Cole, J., Smith, C.M. and Deluca, H.F., 1980. Maternal and fetal serum 1,25-dihydroxyvitamin D levels at term. Journal of Pediatrics 97, 640-642.

Gaultier, C., Harf, A., Balmain, N., Cuisinier-Gleizes, P. and Mathieu, H., 1984. Lung mechanics in rachitic rats. American Review of Respiratory Disease 130, 1108-1110.

Gern, J.E. and Busse, W.W., 2002. Relationship of viral infections to wheezing illnesses and asthma. Nature Reviews: Immunology 2, 132-138.

Gertner, J.M., Glassman, M.S., Coustan, D.R. and Goodman, D.B., 1980. Fetomaternal vitamin D relationships at term. Journal of Pediatrics 97, 637-640.

Godar, D.E., 2001. UV doses of American children and adolescents. Photochemistry and Photobiology 74, 787-793.

Godar, D.E., Pope, S.J., Grant, W.B. and Holick, M.F., 2012. Solar UV doses of young Americans and vitamin D3 production. Environmental Health Perspectives 120, 139-143.

Godar, D.E., Wengraitis, S.P., Shreffler, J. and Sliney, D.H., 2001. UV doses of Americans. Photochemistry and Photobiology 73, 621-629.

Gordon, C.M., Feldman, H.A., Sinclair, L., Williams, A.L., Kleinman, P.K., Perez-Rossello, J. and Cox, J.E., 2008. Prevalence of vitamin D deficiency among healthy infants and toddlers. Archives of Pediatrics & Adolescent Medicine 162, 505-512.

Hansdottir, S. and Monick, M.M., 2011. Vitamin D effects on lung immunity and respiratory diseases. Vitamins and Hormones 86, 217-237.

Hansdottir, S., Monick, M.M., Hinde, S.L., Lovan, N., Look, D.C. and Hunninghake, G.W., 2008. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. Journal of Immunology 181, 7090-7099.

Hansdottir, S., Monick, M.M., Lovan, N., Powers, L., Gerke, A. and Hunninghake, G.W., 2010. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. Journal of Immunology 184, 965-974.

Hiemstra, P.S., 2007. The role of epithelial beta-defensins and cathelicidins in host defense of the lung. Experimental Lung Research 33, 537-542.

Hillman, L.S. and Haddad, J.G., 1974. Human perinatal vitamin D metabolism. I. 25-Hydroxyvitamin D in maternal and cord blood. Journal of Pediatrics 84, 742-749.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Hollis, B.W., 2011. Short-term and long-term consequences and concerns regarding valid assessment of vitamin D deficiency: comparison of recent food supplementation and clinical guidance reports. Current Opinion in Clinical Nutrition and Metabolic Care 14, 598-604.

Hollis, B.W., Johnson, D., Hulsey, T.C., Ebeling, M. and Wagner, C.L., 2011. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. Journal of Bone and Mineral Research 26, 2341-2357.

Hollis, B.W. and Wagner, C.L., 2004a. Assessment of dietary vitamin D requirements during pregnancy and lactation. American Journal of Clinical Nutrition 79, 717-726.

Hollis, B.W. and Wagner, C.L., 2004b. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. American Journal of Clinical Nutrition 80, 1752S-1758S.

Holt, P.G., Strickland, D.H. and Sly, P.D., 2012. Virus infection and allergy in the development of asthma: what is the connection? Current Opinion in Allergy and Clinical Immunology 12, 151-157.

Holt, P.G., Strickland, D.H., Wikstrom, M.E. and Jahnsen, F.L., 2008. Regulation of immunological homeostasis in the respiratory tract. Nature Reviews: Immunology 8, 142-152.

Hypponen, E., Berry, D.J., Wjst, M. and Power, C., 2009. Serum 25-hydroxyvitamin D and IgE – a significant but nonlinear relationship. Allergy 64, 613-620.

Karatekin, G., Kaya, A., Salihoglu, O., Balci, H. and Nuhoglu, A., 2009. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. European Journal of Clinical Nutrition 63, 473-477.

Kovacs, C.S., 2008. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. American Journal of Clinical Nutrition 88, 520S-528S.

Kumar, J., Muntner, P., Kaskel, F.J., Hailpern, S.M. and Melamed, M.L., 2009. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. Pediatrics 124, e362-370.

Laaksi, I., Ruohola, J.P., Tuohimaa, P., Auvinen, A., Haataja, R., Pihlajamaki, H. and Ylikomi, T., 2007. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. American Journal of Clinical Nutrition 86, 714-717.

Lee, J.M., Smith, J.R., Philipp, B.L., Chen, T.C., Mathieu, J. and Holick, M.F., 2007. Vitamin D deficiency in a healthy group of mothers and newborn infants. Clinical Pediatrics 46, 42-44.

Leow, L., Simpson, T., Cursons, R., Karalus, N. and Hancox, R.J., 2011. Vitamin D, innate immunity and outcomes in community acquired pneumonia. Respirology 16, 611-616.

Li-Ng, M., Aloia, J.F., Pollack, S., Cunha, B.A., Mikhail, M., Yeh, J. and Berbari, N., 2009. A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. Epidemiology and Infection 137, 1396-1404.

Litonjua, A.A., 2012. Vitamin D deficiency as a risk factor for childhood allergic disease and asthma. Current Opinion in Allergy and Clinical Immunology 12(2): 179-185.

Looker, A.C., Johnson, C.L., Lacher, D.A., Pfeiffer, C.M., Schleicher, R.L. and Sempos, C.T., 2011. Vitamin D status: United States, 2001-2006. NCHS Data Brief, 1-8.

MacDonald, P.N., Dowd, D.R., Nakajima, S., Galligan, M.A., Reeder, M.C., Haussler, C.A., Ozato, K. and Haussler, M.R., 1993. Retinoid X receptors stimulate and 9-cis retinoic acid inhibits 1,25-dihydroxyvitamin D3-activated expression of the rat osteocalcin gene. Molecular and Cellular Biology 13, 5907-5917.

MacLaughlin, J.A., Anderson, R.R. and Holick, M.F., 1982. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science 216, 1001-1003.

Martinez, F.D., Wright, A.L., Taussig, L.M., Holberg, C.J., Halonen, M. and Morgan, W.J., 1995. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. New England Journal of Medicine 332, 133-138.

McNally, J.D., Leis, K., Matheson, L.A., Karuananyake, C., Sankaran, K. and Rosenberg, A.M., 2009. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatric Pulmonology 44, 981-988.

Morales, E., Romieu, I., Guerra, S., Ballester, F., Rebagliato, M., Vioque, J., Tardon, A., Rodriguez Delhi, C., Arranz, L., Torrent, M., Espada, M., Basterrechea, M. and Sunyer, J., 2012. Maternal vitamin d status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. Epidemiology 23, 64-71.

Muhe, L., Lulseged, S., Mason, K.E. and Simoes, E.A., 1997. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. Lancet 349, 1801-1804.

Nguyen, M., Trubert, C.L., Rizk-Rabin, M., Rehan, V.K., Besancon, F., Cayre, Y.E. and Garabedian, M., 2004. 1,25-Dihydroxyvitamin D3 and fetal lung maturation: immunogold detection of VDR expression in pneumocytes type II cells and effect on fructose 1,6 bisphosphatase. Journal of Steroid Biochemistry and Molecular Biology 89-90, 93-97.

Nguyen, T.M., Guillozo, H., Marin, L., Tordet, C., Koite, S. and Garabedian, M., 1996. Evidence for a vitamin D paracrine system regulating maturation of developing rat lung epithelium. American Journal of Physiology 271, L392-399.

Phokela, S.S., Peleg, S., Moya, F.R. and Alcorn, J.L., 2005. Regulation of human pulmonary surfactant protein gene expression by 1alpha,25-dihydroxyvitamin D3. American Journal of Physiology Lung Cellular and Molecular Physiology 289, L617-626.

Rehman, P.K., 1994. Sub-clinical rickets and recurrent infection. Journal of Tropical Pediatrics 40, 58.

Roth, D.E., Jones, A.B., Prosser, C., Robinson, J.L. and Vohra, S., 2009. Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. European Journal of Clinical Nutrition 63, 297-299.

Roth, D.E., Shah, R., Black, R.E. and Baqui, A.H., 2010. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. Acta Paediatrica 99, 389-393.

Sakurai, R., Shin, E., Fonseca, S., Sakurai, T., Litonjua, A.A., Weiss, S.T., Torday, J.S. and Rehan, V.K., 2009. 1alpha,25(OH)2D3 and its 3-epimer promote rat lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. American Journal of Physiology Lung Cellular and Molecular Physiology 297, L496-505.

Singh, U., Nicholson, G., Urban, B.C., Sargent, I.L., Kishore, U. and Bernal, A.L., 2005. Immunological properties of human decidual macrophages – a possible role in intrauterine immunity. Reproduction 129, 631-637.

Stein, R.T. and Martinez, F.D., 2004. Asthma phenotypes in childhood: lessons from an epidemiological approach. Paediatric Respiratory Reviews 5, 155-161.

Stick, S., 2000. Pediatric origins of adult lung disease. 1. The contribution of airway development to paediatric and adult lung disease. Thorax 55, 587-594.

Stio, M., Celli, A., Lunghi, B., Raugei, G., Modesti, A. and Treves, C., 1997. Vitamin D receptor in IMR-90 human fibroblasts and antiproliferative effect of 1,25-dihydroxyvitamin D3. Biochemistry and Molecular Biology International 43, 1173-1181.

Tavera-Mendoza, L.E. and White, J.H., 2007. Cell defenses and the sunshine vitamin. Scientific American 297, 62-65, 68-70, 72.

UNICEF, 2006. Pneumonia: The Forgotten Killer of children, UNICEF/WHO.

United States Department of Agriculture (USDA-ARS), 2010. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, by Race/Ethnicity and Age, What We Eat in America, NHANES 2007-2008. Available at: www.ars.usda.gov/ba/bhnrc/fsrg.

Urashima, M., Segawa, T., Okazaki, M., Kurihara, M., Wada, Y. and Ida, H., 2010. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. American Journal of Clinical Nutrition 91, 1255-1260.

Walker, V.P., Zhang, X., Rastegar, I., Liu, P.T., Hollis, B.W., Adams, J.S. and Modlin, R.L., 2011. Cord blood vitamin D status impacts innate immune responses. Journal of Clinical Endocrinology and Metabolism 96, 1835-1843.

Wayse, V., Yousafzai, A., Mogale, K. and Filteau, S., 2004. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. European Journal of Clinical Nutrition 58, 563-567.

Whiting, S.J. and Calvo, M.S., 2011. Lifestyle and nutritional determinants of vitamin status. In: D. Feldman, W.J. Pike and J.S. Adams (eds.), Vitamin D. Academic Press, Oxford, UK, pp. 979-1007.

Wright, J.R., 2005. Immunoregulatory functions of surfactant proteins. Nature Reviews: Immunology 5, 58-68.

Wu, P., Dupont, W.D., Griffin, M.R., Carroll, K.N., Mitchel, E.F., Gebretsadik, T. and Hartert, T.V., 2008. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. American Journal of Respiratory and Critical Care Medicine 178, 1123-1129.

Yu, C.K., Sykes, L., Sethi, M., Teoh, T.G. and Robinson, S., 2009. Vitamin D deficiency and supplementation during pregnancy. Clinical Endocrinology 70, 685-690.

Zosky, G.R., Berry, L.J., Elliot, J.G., James, A.L., Gorman, S. and Hart, P.H., 2011. Vitamin D deficiency causes deficits in lung function and alters lung structure. American Journal of Respiratory and Critical Care Medicine 183, 1336-1343.

Key facts

- Vitamin D deficiency is frequent among HIV-infected persons.
- HIV disease or treatment-related factors have been associated with vitamin D deficiency.
- Vitamin D deficiency has been associated with unfavorable HIV disease evolution.
- Causal relationships should be explored in controlled trials.
- Vitamin D supplementation seems effective and safe in HIV-infected persons.

Summary points

- Vitamin D deficiency has been associated, in the general population with conditions that are becoming of concern in HIV-infected persons, which recently generated considerable interest in vitamin D and HIV infection.
- Although vitamin D deficiency does not generally appear more frequent in HIV-infected persons, specific disease-related factors (immune deficiency) and the use of certain antiretroviral drugs have been associated with it in this population.
- Vitamin D deficiency has been associated with long terme complications and comorbidities of treated HIV-infection.
- Vitamin D deficiency has been associated with a pejorative evolution in both untreated and treated Hiv infection.
- Establishing vitamin D deficiency as a new, modifiable cofactor in HIV disease evolution, requires interventional studies.

17. Vitamin D in HIV/AIDS: a role?

J.-P. Viard[1] and J.-C. Souberbielle[2]
[1]Unité Fonctionnelle de Thérapeutique en Immuno-infectiologie, Centre de Diagnostic et de Thérapeutique, Hôtel-Dieu, 1 Place du Parvis Notre-Dame, 751781 Paris Cedex 04, France; [2]Laboratoire d'explorations fonctionnelles, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75783 Paris Cedex 15, France; jean-paul.viard@htd.aphp.fr

Abstract

Vitamin D deficiency is frequent in the general population, where it has been associated with a variety of conditions. While it does not seem to be more frequent in persons living with HIV, factors related to HIV disease or its treatment probably contribute to vitamin D deficiency in this population. Two studies, one in an African, untreated, population, and one in a European population largely treated with antiretrovirals, show that vitamin D deficiency is associated with the onset of clinical events and all-cause mortality in persons living with HIV. The role of vitamin D in T cell activation and the links established between vitamin D deficiency, inflammation, and coagulation activation could explain the clinical associations. These hypotheses should be tested in intervention studies.

Keywords: HIV infection, vitamin D deficiency, antiretroviral drugs, all-cause mortality, vitamin D supplementation

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_17, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
3TC	Lamivudine
AZT	Zidovudine
BMD	Bone mineral density
CI	Confidence interval
CYP450	Cytochrome P450
EFV	Efavirenz
ETV	Etravirine
HAART	Highly active antiretroviral therapy
HCV	Hepatitis C
HR	Hazard ratio
IDV	Indinavir
IQR	Interquartile range
LPV	Lopinavir
NFV	Nelfinavir
NNRTI	Non nucleosidic reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	(HIV) protease inhibitor
PTH	Parathyroid hormone
PY	Patient-years
RPV	Rilpivirine
RTV	Ritonavir
VDR	Vitamin D receptor

17.1 Introduction

Vitamin D is now extensively studied for its extra-osseous effects. $1,25(OH)_2D$ is a steroid hormone that binds a cytosolic receptor regulating gene function in many different cell types, including innate and adaptive immunity cells. Mice in which the VDR gene has been invalidated present with multiple dysfunctions (Bouillon *et al.*, 2008) and, in humans, vitamin D deficiency has been epidemiologically associated, in the general population, with a wide variety of conditions: infections (particularly tuberculosis), cancers (particularly breast and colon cancers), coronary artery disease and hypertension, insulin resistance and diabetes, and autoimmune diseases (Souberbielle *et al.*, 2010). In addition, in older persons or in patients with chronic condition (diabetes, high cardiovascular risk, cancer, heart transplant, renal failure, etc.) vitamin D deficiency has been associated with a higher risk of all-cause or cardiovascular death (Dobnig *et al.*, 2008).

Considering first the frequency of vitamin D deficiency in the general population, second the frequency of lower bone mineral content in the HIV-infected population (Childs *et al.*, 2011), and third the large overlap between vitamin D-associated conditions (in the general population) and the emerging long-term comorbidities/complications of antiretroviral-treated HIV infection, the question of the relationships between vitamin D status and HIV infection had to be addressed (Giusti *et al.*, 2011). Five main issues can be raised: (1) What is the frequency of vitamin D deficiency in HIV-infected persons? (2) Are there specific disease- or treatment-related factors associated with vitamin D deficiency in HIV-infected persons? (3) Is vitamin D status associated with HIV disease course or complications? (4) If yes, what could be the reasonable explanations and testable hypotheses to demonstrate causal relationships? (5) Is vitamin D supplementation warranted in persons living with HIV?

17.2 Vitamin D insufficiency in HIV-infected persons

Vitamin D insufficiency or deficiency is widespread in the general population, and its prevalence has augmented over the last decade, in men and women, across age and ethnic categories, which has been mainly explained by changes in life style and by the increased prevalence of obesity. In the US, the prevalence of severe deficiency (25(OH)D <10 ng/ml) has increased from 2% to 6% from 1988-1994 to 2001-2004 (Ginde *et al.*, 2009). In Europe, general population studies found severe deficiency in 2-30% of adults (Mithal *et al.*, 2009). In cross-sectional studies of HIV-infected persons, the prevalence of vitamin D insufficiency (25(OH)D <30 ng/ml) varied from 39.3% in Tanzania (Mehta *et al.*, 2010) to 93% in France (Legeai *et al.*, 2012); the prevalence of levels <20 ng/ml varied from 26.8% in Thailand (Wiboonchutikul *et al.*, 2012) to 67% in France (Legeai *et al.*, 2012); and the prevalence of severe deficiency varied between 0% in Hawaii (Shikuma *et al.*, 2012), and 42% in Switzerland during Spring (Mueller *et al.*, 2010).

Studies have compared 25(OH)D levels in HIV-infected and HIV-negative persons (Table 17.1). Studies in the US found that vitamin D deficiency was not more prevalent in HIV-positive patients than in control populations. For example, the prevalence of a 25(OH)D level below 30 ng/ml and below 20 ng/ml were comparable in the SUN cohort (HIV-infected persons) and in the NHANES study (general population) after adjusting for age, gender and race: 70.3% vs. 79.1% and 29.7% vs. 38.8%, respectively (Dao *et al.*, 2011). Vitamin D deficiency therefore appeared rather less prevalent in HIV-infected persons. This could be explained by a smaller body mass index in HIV-infected patients, or by the fact that persons belonging to certain HIV transmission groups may have life styles leading to more sun exposure, or by the fact that being under care for HIV infection may drive people to pay more attention to nutritional efforts, particularly in countries where dairy products are enriched in vitamin D. Two studies, one from Spain (Cervero Jimenez *et al.*, 2012) and one from India (Paul *et al.*, 2010), found vitamin D deficiency to be more prevalent in HIV-infected persons.

Patients enrolled in these studies (Table 17.1) were largely receiving antiretroviral drugs. Fewer studies have been published in untreated HIV-infected persons. Indications can be found in

Table 17.1. Prevalence of vitamin D deficiency in cross-sectional studies of HIV-infected persons vs. HIV-negative controls.

Study	Country	Number of participants			25(OH)D thresholds	Participants with vitamin D deficiency		
		HIV-positive		HIV-negative		HIV-positive		HIV-negative
		Number	On anti-retrovirals					
Ross *et al.*, 2011	USA (Ohio)	149	100%	39		Mean: 52.1±1.04 nmol/l		Mean: 64.6±1.08 nmol/l
					>30ng/ml	21%		32%
					>20 ng/ml	54%		71%
					>10 ng/ml	95%		100%
Milazzo *et al.*, 2011	Italy	144 with HIV, 93 with HIV/HCV	100%	76, age, gender and BMI-matched		HIV	HIV/HCV	
					<10-30 ng/ml	63.2%	59%	75%
					<10 ng/ml	7.46%	4.3%	1.3%
Dao *et al.*, 2011	USA (different states)	672	89.9%	US general populations (NHANES study)	<30 ng/ml	70.3%		79.1%
					<20 ng/ml	29.7%		38.8%
Cervero Jimenez *et al.*, 2012	Spain	352	89.5%	116 (prior study)	<30 ng/ml	71.6%		83.6%
					<20 ng/ml	44.0%		27.6% (prevalence significantly lower)
Rutstein *et al.*, 2011	USA (PA)	81 children (mean age: 13.8±4.1)	84%	372 healthy children (mean age: 12.4±3.4)	<30 ng/ml	11%		16%
					11-29 ng/ml	45%		69%
					<11 ng/ml	36%		15%
Stein *et al.*, 2011	USA (NY)	89 post-menopausal African American and Hispanic women	80%	94 post-menopausal African American and Hispanic women	<30 ng/ml	74.0%		78.0%
Adeyemi *et al.*, 2011	USA (different states)	1,268 women (WIHS study)	72%	510 women (WIHS study)	<20 ng/ml	60.0%		72.0%

Table 17.1. Continued.

Study	Country	Number of participants			25(OH)D thresholds	Participants with vitamin D deficiency	
		HIV-positive		HIV-negative		HIV-positive	HIV-negative
		Number	On anti-retrovirals				
Yin *et al.*, 2010	USA (different states)	82 pre-menopausal women	56%	58 pre-menopausal women	<32 ng/ml	91%	91%
					<20 ng/ml	69%	60%
					<10 ng/ml	30%	24%
Paul *et al.*, 2010	India	70 men	50%	35 matched men	<20 ng/ml	treated : 74% naïve: 37%	37%
Ormesher *et al.*, 2011	USA (DC)	199 African-American men	Not reported	424 matched African-American men (NHANES Study)	<30 ng/ml	77.4%	96.2%

a study of untreated Tanzanian women (Mehta *et al.*, 2010) and in baseline data of cohorts or trials initiating antiretroviral therapy (Table 17.2). These data are important because antiretroviral drugs may have an impact on vitamin D metabolism.

17.3 Factors associated with vitamin D insufficiency in HIV-infected persons

Although vitamin D deficiency does not generally appear more prevalent in HIV-infected persons than in the general population, specific disease- or treatment-related factors have been associated with low 25(OH)D levels in this group, in addition to classical risk factors.

Studies (generally multivariate analyses) do not all agree on the factors associated with lower 25(OH)D levels in HIV-infected persons, some of them even present contradictory results. The following traditional risk factors have been evidenced in several studies: older age (Dao *et al.*, 2011; Rutstein *et al.*, 2011; Viard *et al.*, 2011), black ethnicity and season of sampling (Adeyemi *et al.*, 2011; Brown *et al.*, 2010; Cervero Jimenez *et al.*, 2012; Conesa-Botella *et al.*, 2010; Choi *et al.*, 2011; Crutchley *et al.*, 2011; Dao *et al.*, 2011; Kim *et al.*, 2011; Kwan *et al.*, 2012; Mueller *et al.*, 2010; Rutstein *et al.*, 2011; Shikuma *et al.*, 2011; Stein *et al.*, 2011; Van den Bout *et al.*, 2008; Vescini *et al.*, 2011; Viard *et al.*, 2011; Welz *et al.*, 2010), geographical region (Viard *et al.*, 2011). In line with what has been observed in the general population, one study found recent samples to be more at risk for low 25(OH)D than older samples (Viard *et al.*, 2011). In a majority of studies, higher body mass index was associated with higher odds of vitamin D deficiency (Adeyemi *et al.*, 2011; Cervero Jimenez *et al.*, 2012; Crutchley *et al.*, 2011; Choi *et al.*, 2011; Dao *et al.*, 2011), but one found an association with higher 25(OH)D levels. This discrepancy may reflect a complex relationship between 25(OH)D levels, adipose tissue volume and the interference of some antiretrovirals with adipocyte metabolism (lipodystrophy syndrome). Among HIV-related factors, the route of HIV transmission (namely non homosexual, or intravenous drug use) (Mueller *et al.*, 2010; Viard *et al.*, 2011), the current CD4 count or the CD4 count nadir (i.e. the lowest value ever reached by a given individual) (Adeyemi *et al.*, 2011; Kwan *et al.*, 2012; Rutstein *et al.*, 2011; Shikuma *et al.*, 2011; Stein *et al.*, 2011) and the exposure to antiretroviral treatment (Allavena *et al.*, 2012; Paul *et al.*, 2010; Welz *et al.*, 2010) have been reported. Contradictory results exist as regards the association with detectable (Cervero Jimenez *et al.*, 2011; Kim *et al.*, 2011; Kwan *et al.*, 2012) vs. undetectable (Adeyemi *et al.*, 2011) plasma HIV RNA viral load, which may be related to treatment history.

It should be noted that the 'factors associated with vitamin D deficiency' or 'predictors of vitamin D deficiency' reported in these studies may in fact fall into two categories: some of these factors (e.g. anthropometric, sociodemographic and geographic characteristics, antiretroviral treatment use) are plausible explanatory factors for low 25(OH)D levels, from a mechanistic point of view, while others (i.e. current or nadir CD4 count, viral load) could either be explanatory factors on the path to vitamin D deficiency or a consequence thereof. In the latter category, metabolic parameters and end-organ abnormalities, will be discussed below.

Table 17.2. Vitamin D deficiency in antiretroviral-naïve HIV-infected persons, longitudinal studies and clinical trials.

Study	Type of study (follow-up)	Country	Number of participants	Antiretroviral-naïve at baseline	Antiretroviral regime	25(OH)D thresholds	Participants with vitamin D deficiency at baseline	Evolution of 25(OH)D
Brown and McComsey, 2010	Longitudinal (6-12 months)	USA (Ohio)	87	100%	EFV-based (n=51); non-EFV-based (n=36)	<37.5 nmol/l	33%	Decrease in EFV group: -12,7 (IQR:-20; 2.7) nmol/l Stable in non-EFV group: 1.0 (IQR: -10.2; 14.5) nmol/l
Conesa-Botella *et al.*, 2010	Longitudinal (12 months)	Belgium	87	100%	NNRTI-based (n=43; EFV=20, NVP=23);	<30 ng/ml	70.1%	Decrease of (25)OHD: 81.6%
					PI-based (n=44)	<20 ng/ml	43.7%	47.1%
Lattuada *et al.*, 2009	Longitudinal (3 years)	Italy	18	100%	Starting AZT+3TC+NVP	<25 nmol/ll	12%	No significant evolution (means): baseline: 52.0±28.1 nmol/l year 3: 66.1±21.0 nmol/l
Mueller *et al.*, 2010	Longitudinal (18 months)	Switzerland	211	100%	Mixed			No change on HAART:
						>75 nmol/l	5% (spring)	10% (spring)
							24% (autumn)	23% (autumn)
						30-74 nmol/l	53% (spring)	38% (spring)
							62% (autumn)	59% (autumn)
						<30 nmol/l	42% (spring)	52% (spring)
							14% (autumn)	18% (autumn)
Rockstroh *et al.*, 2010	Clinical trial (24 weeks)	Europe	157	100%	2 nucleosides plus etravirine or EFV	<50 nmol/l	26%	Mean 25(OH)D at week 0 and 24: EFV: 62.4 and 41.4 nmol/l ETV: 64.7 and 44.4 nmol/l
						<25 nmol/l	2.5%	

Table 17.2. Continued.

Study	Type of study (follow-up)	Country	Number of participants	Antiretroviral-naïve at baseline	Antiretroviral regime	25(OH)D thresholds	Participants with vitamin D deficiency at baseline	Evolution of 25(OH)D
Wohl *et al.*, 2011	Clinical trial (48 weeks)	World	582	100%	Tenofovir/ emtricitabine plus EFV or RPV	<75 nmol/l 25-50 nmol/l <25 nmol/ml	67.0% 24.7% 4.6%	Week 48 decrease: EFV: -6.2±18.0 nmol/l RPV: -0.6±17.9 nmol/l 25(OH)D <25 nmol/l at week 48: EFV:9.0% RPV: 4.5%
Van Vonderen *et al.*, 2009	Clinical trial	Europe, Russia, Israel	48	100%	AZT/3TC/LPV/RTV or NVP/LPV/RTV	Not reported	Not reported	No change over time in either group
Legeai *et al.*, 2012	Cohort	France	355	100%	Mixed	<30 ng/ml <20 ng/ml <10 ng/ml	93% 67% 24%	Not reported yet

The possible impact of antiretroviral drugs on vitamin D metabolism is an intriguing issue, with some conflicting data between *in vitro* and *in vivo* studies. Several reports, including longitudinal studies (Table 17.2), have shown an independent association of lower or decreasing 25(OH)D concentrations with the exposure to EFV, and not NVP (Lattuada *et al.*, 2009) or ETV (Rockstroh *et al.*, 2010) or RPV (Wohl *et al.*, 2011), all four drugs belonging to the NNRTI class. All studies, including longitudinal studies, have shown a neutral, or even protective effect of PI use on vitamin D deficiency, as reflected by blood 25(OH)D levels. One study (Fox *et al.*, 2010) has suggested that being on the nucleosidic reverse transcriptase inhibitor (NRTI) zidovudine was also a risk factor for deficiency, and that stopping this drug was associated with increasing 25(OH)D with time, just as was the case with EFV.

Why should antiretrovirals influence 25(OH)D levels? Several of them are substrates of, and have an impact on, the CYP450 enzyme complex, particularly on the CYP3A4 isoenzyme (Tseng and Foisy, 2012). For instance, NNRTIs are CYP3A4 inducers, while PIs are CYP3A4 inhibitors. This is especially true for the PI RTV, probably the most potent CYP3A4 inhibitor among licensed drugs, which is used at a low, virologically inefficient, dosage as a pharmacological booster for the other PIs. The metabolism of vitamin D relies on hydroxylations through CYP450 enzymes (Holick, 2007): activation through 25- and 1α-hydroxylations (particularly by CYP2R1, CYP27B1), and inactivation through 24- and 4-hydroxylations (by CYP24 and CY3A4). PIs and NNRTIs could therefore modify vitamin D metabolism, the hypothesis being that PIs would inhibit vitamin D activation and NNRTIs would increase its inactivation, since CYP3A4 has been shown to play a direct role in 24-and 4-hydroxylation (Wang *et al.*, 2012; Yang *et al.*, 2006).

As shown in Table 17.3, it has indeed been shown that 'first generation' PIs (IDV, NFV, RTV) were able to impair 25(OH)D3 synthesis in hepatocyte and 1,25(OH2D3 synthesis in monocyte cell line cultures, in a reversible, dose-dependent manner (Cozzolino *et al.*, 2003). PIs also inhibited 24-hydroxylation in macrophages, but with a lower potency than that shown on 1α-hydroxylation, resulting in a net reduced 1,25(OH)D3 production in these cells. It has also been shown that, among NNRTIs, both EFV and NVP were able to increase expression of the VDR and the vitamin D-induced upregulation of the CYP24 gene, in human renal clear-cell carcinoma cells (Landriscina *et al.*, 2008). EFV was also shown to suppress the expression of CYP2R1 in fibroblasts (Ellfolk *et al.*, 2009) (Table 17.4).

Data on 1,25(OH)2D are few but they may provide insight in the impact of antiretrovirals on vitamin D metabolism. In the Swiss cohort study, no correlation with NNRTI use was found, but tenofovir use was correlated with higher 1,25(OH)2D, as was body mass index, while HCV seropositivity, previous AIDS and high CD4 counts were correlated with lower $1,25(OH)_2D$ (Mueller *et al.*, 2010). To date, no study examining the effects of antiretroviral drugs on the levels of vitamin D inactive metabolites (e.g. 24- or 4-hydroxylated forms) has been published. This would be of high interest, since there is some discrepancies between the few *in vitro* studies and the already numerous clinical studies, only based on 25(OH)D measurement (e.g. effect of PIs, differences between NNRTIs).

Table 17.3. Documented effects of antiretroviral drugs on vitamin D metabolism *in vitro*.

Study	Drugs tested	Cell model	Effects	Comments
Cozzolino *et al.*, 2003	RTV, IDV, NFV	THP-1 monocyte cell line-derived macrophages	Inhibition of 1a-hydroxylase (RTV, IDV, NFV) Inhibition of 24-hydroxylase (RTV, UIDV, NFV) Net effect: decreased 1,5(OH)2D in macrophages	EFV shows no effect
		Hep3B hepatocyte cell line	Inhibition of 25-hydroxylase	
Landriscina *et al.*, 2008	EFV, NFV	Clear-cell renal carcinoma primary culture	Upregulation of VDR and calbindin 28k genes Enhanced expression of CYP24 in response to vitamin D	
Ellfolk *et al.*, 2009	EFV	BJ dermal fibroblast cell line	60% decrease of CYP2R1 mRNA 30% reduction of 1,25(OH)D synthesis	No effect on CYP27A1 EFV and 1,25$(OH)_2$D had similar effects
		LNCaP prostate cancer cell line	25% decrease of CYP2J2 mRNA No effect on CYP2R1	No pregnance X receptor expression on LNCap, may explain differences with BJ

Table 17.4. Incidence rate of events according to 25(OH)D tertile (ng/ml) at baseline in 1,985 patients fom the EuroSIDA Cohort (per 100 PY of follow-up) (Viard *et al.*, 2011).

Events		Total	25(OH)D≤12	12<25(OH)D≤20	25(OH)D>20
AIDS-defining events	No. of events	159	73	39	47
	PY	11,720	3,737	3,654	4,329
	Incidence (95% CI)	1.36 (1.15-1.87	1.95 (1.51-2.40)	1.07 (0.73-1.40)	1.09 (0.78-1.40)
All-cause deaths	No. of events	188	87	47	54
	PY	12,225	3,963	3,780	4,482
	Incidence (95% CI)	1.54 (1.32-1.76)	2.20 (1.73-2.66)	1.24 (0.89-1.60)	1.20 (0.88-1.53)

The impact of tenofovir, a NRTI has also been examined because it can induce proximal tubulopathy with an increased urinary excretion of phosphorus, which could in turn modify the metabolism of vitamin D. Exposure to tenofovir has not been found to be a risk factor for lower 25(OH)D levels, and some studies even suggested a protective role (Allavena *et al.*, 2012; Dao *et al.*, 2011). There seems to be a complex interaction between tenofovir exposure and vitamin D deficiency. Persons on tenofovir who also have vitamin D deficiency are those showing the highest alkaline phosphatase and PTH levels (Childs *et al.*, 2010; Pocaterra *et al.*, 2011; Rosenvinge *et al.*, 2010). Vitamin D supplementation can lower PTH levels in persons on tenofovir, more efficiently than in those receiving other NRTIs (Havens *et al.*, 2012).

17.4 Vitamin D and long-term non-AIDS defining complications and comorbidities

Due to the efficacy of antiretroviral combinations, HIV infection has become a chronic disease characterized by a life-long treatment and by the emergence of conditions that are considered as age-related in the general population, such as non AIDS-defining cancers (e.g. lung cancer), metabolic disorders (hyperlipemia, insulin resistance and diabetes), cardiovascular disease, osteopenia and osteoporosis, renal impairment, neurocognitive disorders, etc. These conditions appear with a higher incidence, and at an earlier age, in the HIV-infected population than in the general population. This has led to seeing HIV-infected persons as experiencing an 'accelerated aging' phenomenon. It is not easy to define the respective responsibility of harbouring the retrovirus (even on antiretrovirals) and of being exposed to drugs (with a toxic potential) for decades, in the pathogenesis of these complications and comorbidities. However, since vitamin D deficiency has been epidemiologically associated with many of these conditions in the general populations, it is no surprise that studies were performed to test whether these associations also hold true in HIV-infected persons.

As reviewed in 2011 (Childs *et al.*, 2011), osteopenia (BMD T-score between -1 and -2.5) and osteoporosis (T-score<-2.5) are frequent in persons living with HIV: they were found respectively in 52% and 15% of patients in a meta-analysis including studies published up to 2005 (Brown and Qaqish, 2006). In a French study that carefully excluded patients with a possible cause for secondary BMD loss (Mary-Krause *et al.*, 2009), among 700 men and 192 women, a T-score ≤2.5 was found in 7.9% of men and 1.1% of women, with a median age of 46 years (IQR, 41-53). In these studies, classical risk factors for bone loss (low body mas index, older age, glucorticoid use) were found to be predictors, but some HIV-related characteristcs also emerged, such as duration of HIV infection, CD4 count nadir, previous AIDS-defining conditions, and antiretroviral use (particularly PI use and recent tenofovir exposure). Although not clearly associated with low BMD in all cross-sectional studies (Childs *et al.*, 2011), lower 25(OH)D has been associated with lower hip BMD at baseline and with greater BMD reduction at the femoral neck during prospective follow-up (Dolan *et al.*, 2006; Yin *et al.*, 2010).

Preliminary results in HIV-infected persons indicate that vitamin D deficiency is associated with type 2 diabetes (Szep *et al.*, 2011) and insulin resistance (Hammond *et al.*, 2012), carotid intima-media thickness (Choi *et al.*, 2011; Ross *et al.*, 2011), arterial dysfunction, namely coronary calcification and flow-mediated vasodilation (Shikuma *et al.*, 2011), proteinuria in injection drug users (Estrella *et al.*, 2012), and with a composite endpoint of non-AIDS defining conditions (chronic kidney disease, diabetes, cardiovascular events, non-AIDS defining malignancies) (Vescini *et al.*, 2011). Vitamin D deficiency has also been associated with the severity of liver fibrosis in HIV/HCV coinfected persons with active chronic hepatitis (Terrier *et al.*, 2011). Few of these studies examined correlations of vitamin D status in HAART-naïve patients, which is a problem because antiretrovirals improve immune functions, decrease inflammation to a certain extent, and because some drugs have a negative impact on inflammation, lipid levels, glucose homeostasis, adipocyte and kidney proximal tubule functions. All this makes it difficult to examine the potential impact of vitamin D deficiency independently of the positive or negative effects of drugs. One large study in antiretroviral naïve patients (Legeai *et al.*, 2012) showed an association of low 25(OH)D with lower CD4 counts, higher inflammation markers and lipid parameters (lower low-density lipoproteins-cholesterol), with noticeable differences between black and white patients. Several studies have found a negative association between 25(OH)D and estimated glomerular filtration rate in HIV-infected persons, treated or not (which demonstrates that this is not related to drug toxicity): this intriguing finding may be due to an increase in creatinine production through VDR activation, without modification of creatinine or iothalamate clearance, as suggested in a study where patients with chronic kidney disease received paricalcitol (Agarwal *et al.*, 2011).

17.5 Vitamin D insufficiency and HIV disease clinical progression

More surprisingly, vitamin D deficiency has been associated with a defavourable evolution of HIV disease itself, both in antiretroviral-naïve patients and in the setting of widespread antiretroviral treatment.

In a secondary analysis of a study performed in 1995-1997 in 884 untreated HIV-infected pregnant Tanzanian women, 25(OH)D was measured at enrolment. A median prospective follow-up of 70 months, without vitamin D supplementation was available for the analysis of events (Mehta *et al.*, 2010). 347 women had a 25(OH)D below 32 ng/ml. Compared to those with a level equal or superior to 32 ng/ml, they showed a higher risk of clinical progression, according to the WHO classification, with a HR of 1.25 (95% CI: 1.05-1.50) and a higher risk of anemai, with a HR of 1.46 (95% CI: 1.09-1.96). Furthermore, having a 25(OH)D level in the highest quintile, compared to the lowest quintile was associated with a lower risk of all-cause death, with a HR of 0.58 (95% CI: 0.40-0.84). Women with low vitamin D also had a higher risk of developing wasting syndrome, oral thrush, and acute upper respiratory tract infections (Mehta *et al.*, 2011). The 25(OH)D level in mothers was also associated with a higher risk of morbidity and mortality in children (Mehta *et al.*, 2009). Those born from mothers with a 25(OH)D level below 32 ng/ml had a higher risk of mother-to-child HIV transmission at 6 weeks (HR: 1.50, 95% CI: 1.02-2.20) and during

breastfeeding (HR: 2.03, 95% CI: 1.08-3.82). They also had a higher risk of reporting cough during follow-up, of stunting and being underweight (Finkelestein *et al.*, 2012), and of all-cause death at 24 months (HR: 1.61, 95% CI: 1.25-2.07).

In the EuroSIDA cohort study (Viard *et al.*, 2011), 25(OH)D was measured in 1,985 patients at enrolment and a median 5-year prospective follow-up was available for the analysis of events. 83% of patients were on antretroviral treatment. A strong and robust association between having a baseline 25(OH)D level in the lowest tertile (<12 ng/ml) and the occurence of AIDS-defining events and all-cause deaths was found. Persons with baseline 25(OH)D levels in the midlle and higher tertiles had a 40% lower incidence rate ratio for these endpoints, which was statistically significant in a multivariate analysis adjusted for demographic characteristics, calendar year, season, geographical region, CD4 count, HIV ARN plasma load and antiretroviral treatment (Table 17.4).

There are major differences between the two studies mentioned above: untreated vs. treated HIV infection, African vs. European setting, nutritional status, and socio-economic level of persons. This raises the interesting question of the relevant threshold that would define a higher risk of HIV disease clinical evolution: in the Tanzanian study, women with a 25(OH)D just below the 'optimal' level appeared at a higher risk, while in the EuroSIDA study, the risk was clearly associated with a severely deficient status. One could hypothesize that in the most vulnerable patients (malnourished, with multiple nutriment deficiencies and with no antiretroviral treatment), mild vitamin D insufficiency is sufficient to accelerate disease evolution, while in a more favourable setting, only severe deficiency becomes a cofactor of disease progression.

It should be emphasized that these results, obtained from observational studies, do not allow to draw any firm conclusion on the causal relationship between vitamin D insufficiency or deficiency and the occurrence of events. However, the results appear striking enough as to view vitamin D deficiency as a possible, hitherto unrecognized, cofactor in HIV disease evolution.

17.6 Testable hypotheses towards establishing causal relationships

In the setting of HIV infection, just like in the general population, the key question is the following: how can vitamin D deficiency be associated with such a wide range of conditions? Two series of hypotheses seem particularly interesting in this context, and could be tested in controlled trials, in order to explore causal relationships.

The first one is the role played by vitamin D in the regulation of immune responses. Vitamin D could be instrumental in first-line, innate immunity against a variety of pathogens, including HIV itself. Thus, a study has clearly shown its role in the induction of autophagy (Campbell *et al.*, 2011). Autophagy is a finely regulated mechanism that is important in the defence against infectious agents, particularly viruses, and which is inhibited by HIV. Active vitamin D induced autophagy in monocyte-derived macrophages, in a dose-dependent manner. Moreover, this was associated

with a reduced infectability of the cells with *Mycobacterium tuberculosis* and HIV. It has been also shown that the vitamin D – VDR system is necessary to the TCR-mediated activation of naïve T cells (Von Essen *et al.*, 2010). One can therefore hypothesize that vitamin D deficiency contributes to the abnormalities of cell-mediated immunity that persist in patients, even when they receive fully suppressive antiretroviral drugs (residual immune activation, immune senescence, etc.). Before the active antiretroviral therapy era, low 1,25(OH)D2 has been associated with immune deficiency and poorer survival (Haug *et al.*, 1994). Vitamin D deficiency has also been associated with a smaller CD4 cell gain on antiretroviral therapy (Ross *et al.*, 2011). Whether vitamin D administation improves innate immune functions or CD4 counts and functions in HIV-infected persons should be tested.

The second series of hypotheses pertains to the association between vitamin D deficiency on the one hand, and, on the other hand, the activation of inflammation and coagulation. In patients addressed for a coronary angiography, vitamin D deficiency was associated with mortality but also with high levels of cell adhesion, oxidative stress and inflammation markers (in particular C-reactive protein and interleukin 6) (Dobnig *et al.*, 2008). In a placebo-controlled study (Schleithoff *et al.*, 2006), vitamin D supplementation allowed a significant reduction in the serum level of TNF-α et and a significant rise of the anti-inflammatry cytokine IL-10. In a large general population cohort, low vitamin D levels were associated with higher levels of markers of pre-clinical activation of coagulation (tissue activator of plasminogen and D-dimers) (Hyppönen *et al.*, 2010). This makes particular sense in the setting of HIV infection, because inflammation (CRP and IL-6) and coagulation activation (D-dimers) markers have been proposed as predictors of mortality and opportunistic diseases in HIV-infected patients (Kuller *et al.*, 2008, Rodger *et al.*, 2009). Whether vitamin D administration can lower the level of these and other biomarkers of immune activation, inflammation, and coagulation, should also be tested.

17.7 Vitamin D supplementation in HIV-infected persons

A study of 483 HIV-infected patients (93% on antiretrovirals) tested vitamin D supplementation (Pacanowski *et al.*, 2012): median baseline 25(OH)D was 19 ng/ml (IQR: 13-26), 25(OH)D was <30 ng/ml in 81% and <10 ng/ml in 12% of persons, and vitamin D3 (100,000 IU) was administered monthly. After 4 months, median 25(OH) rised to 36 ng/ml (IQR: 28-43) and 70% of patients reached a 25(OH)D level in the desirable range (≥30 ng/ml). Risk factors for a persistant suboptimal level (<30 ng/ml) at month 4 were a low actual supplementation dosage (<300,000 IU), a low 25(OH)D baseline level (<30 ng/ml), non-white ethnicity, a high body mass index (>25), and being on the NRTI zidovudine. Vitamin D supplementation was well tolerated with no report of hypercalcemia.

Foissac *et al.* (2012) performed a population pharmacokinetic study, modeling optimal supplementation from observational data of 422 HIV-infected patients starting with a median 25(OH)D of 16 ng/ml (range: 11-23). In order to reach a 25(OH)D level in the 30 to 150 ng/ml

range in 90% of patients, they proposed a dosing scheme of 100,000 IU every month. This scheme resulted in less than 1% of patients above 100 ng/ml 25(OH)D.

17.8 Conclusions

The ultimate question of interest to all clinicians involved in HIV-infected patients care is: 'should patients with vitamin D deficiency be supplemented?' In our opinion the answer to this difficult question is twofold.

On the one hand, there is no definitive proof that vitamin D deficiency is causal in the complications of HIV disease. In consequence, with the exception of bone mineral density abnormalities, for which the scheme defined in the general population can reasonably be superimposed to HIV-infected persons, there is no hard evidence that vitamin D supplementation will improve health outcomes in this population.

On the other hand, vitamin D deficiency is frequent in people living with HIV and they resemble, in many aspects, persons weakened by age or underlying conditions such as diabetes, high vascular risk, chronic kidney disease, etc, all settings were vitamin D deficiency has been associated with a higher risk of complications and mortality. Therefore, without any proof of causal relationships, and without any 25(OH)D target value linking vitamin D status with the onset of specific outcomes, we would recommend supplementation, without prior measurements (except when bone disease is diagnosed). According to practice, availability of vitamin D formulations, and patients' preferences, daily doses of vitamin D2 or 3 around 3,000 UI/day or monthly doses of vitamin D3 around 100,000 IU (possibly after a more intense dosing during the first 6 weeks in patients with severe deficiency) seem adequate.

References

Adeyemi, O.M., Agniel, D., French, A.L., Tien, P., Weber, K., Glesby, M.J., Villacres, M.C., Sharma, A., Merenstein, D., Golub, E.T., Meyer, W. and Cohen, M., 2011. Vitamin D deficiency in HIV-infected and uninfected women in the US. Journal of Acquired Immune Deficiency Syndromes 57, 197-204.

Agarwal, R., Hynson, J.E., Hecht, T.J., Light, R.P. and Sinha, A.D., 2011. Short-term vitamin D receptor activation increases serum creatinine due to incraesed production with no effect on the glomerular filtration rate. Kidney International 80, 1073-1079.

Allavena, C., Delpierre, C., Cuzin, L., Rey, D., Viget, N., Bernard, J., Guillot, P., Duvivier, C., Billaud, E. and Raffi, F., 2012. High frequency of vitamin D deficiency in HIV-infected patients: effects of HIV-related factors and antiretroviral drugs. Journal of Antimicrobial Chemotherapy 67, 2222-2230.

Bouillon, R., Carmeliet, G., Verlinden, L., van Etten, E., Verstuyf, A., Luderer, H.F., Lieben, L., Mathieu, C. and Demay, M., 2008. Vitamin D and human health: lessons froms vitamin D receptor null mice. Endocrine Reviews 29, 726-776.

Brown, T.T. and McComsey, G.A., 2010. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. Antiviral Therapy 15, 425-429.

Brown, T.T. and Qaqish, R.B., 2006. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 20, 2165-2174.

Campbell, G.R. and Spector, S.A., 2012. Vitamin D inhibits human immunodeficiency virus type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. PLoS Pathogens 8(5): e1002689.

Cervero Jimenez, M., Agud, J.L., Garcia-Lacalle, C., Alcazar, V., Torres, R., Jusdado, J.J. and Moreno, S., 2012. Prevalence of vitamin D deficiency and its related risk factors in a Spanish cohort of adult HIV-infected patients: effects of antiretroviral therapy. AIDS Research and Human Retroviruses 28, 963-971.

Childs, K., Welz, T., Samrawickrama, A. and Post, F.A., 2012. Effects of vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. AIDS 26, 253-262.

Childs, K.E., Fishman, S.L., Constable, C., Gutierrez, J.A., Wyatt, C.M., Dieterich, D.T., Mullen, M.P. and Branch, A.D., 2010. Inadequate vitamin D exacerbates parathyroid hormone elevations in tenofovir users. AIDS Research and Human Retroviruses 26, 855-859.

Choi, A.I., Lo, J.C., Mulligan, K., Schnell, A., Kalapus, S.C., Li, Y., Hunt, P.W., Martin, J.N., Deeks, S.G. and Hsue, P.Y., 2011. Association of vitamin D insufficiency with carotid intima-media thickness in HIV-infected persons. Clinical Infectious Diseases 52, 941-944.

Conesa-Botella, A., Florence, E., Lynen, L., Colebunders, R., Menten, J. and Moreno-Reyes, R., 2010. Decrease of vitamin D concentration in patients with HIV infection on a non nucleoside reverse transcriptase inhibitor-containing regimen. AIDS Research and Therapy 7, 40-46.

Cozzolino, M., Vidal; M., Arcidiacono; M.V., Tebas, P., Yarasheski, K. and Dusso, A.S., 2003. HIV-protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. AIDS 17, 513-520.

Crutchley, R.D., Gathe, J., Mayberry, C., Trieu, A., Abughosh, S. and Garey, K.W., 2012. Risk factors for vitamin D deficiency in HIV-infected patients in the South Central United States. AIDS Research and Human Retroviruses 28, 454-459.

Dao, C.N., Patel, P., Overton, E.T., Rhame, F., Pals, S.L., Johnson, C., Bush, T. and Brooks, J.T., 2011. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. Clinical Infectious Diseases 52, 396-405.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., Kinkeldei, J., Boehm, B.O., Weihrauch, G. and Maerz, W., 2008. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Archives of Internal Medicine 168, 1340-1349.

Dolan, S., Kanter, J.R. and Greenspoon, S., 2006. Longitudinal analysis of bone density in human immunodeficiency virus-infected women. Journal of Clinical Endocrinology and Metabolism 91, 2938-2945.

Ellfolk, M., Norlin, M., Gyllensten, K. and Wikvall, K., 2009. Regulation of human vitamin D3 25-hydroxylases in dermal fibroblasts and prostate cancer LNCaP cells. Molecular Pharmacology 75, 1392-1399.

Estrella, M.M,. Kirk, G.D., Mehta, S.H., Brown, T.T., Fine, D.M., Atta, M.G. and Lucas, G.M., 2012. Vitamin D deficiency and persistent proteinuria among HIV-infected and uninfected injection drug users. AIDS 26, 295-302.

Finkelstein, J.L., Mehta, S., Duggan, C., Manji, K.P., Mugusi, F.M., Aboud, S., Spiegelman, D., Msamanga, G.I. and Fawzi, W.W., 2012. Maternal vitamin D status and child morbidity, anemia, and growth in Human immunodeficeincy virus-exposed children in Tanzania. Pediatric Infectious Diseases 31, 171-175.

Foissac, F, Tréluyer, J.M., Rostane, H., Urien, S. and Viard, J.P., 2012. Vitamin D3 supplementation scheme in HIV-infected patients based upon a pharmacokinetic modelling of 25-hydroxycholecalciferol. British Journal of Clinical Pharmacology. Available at http://dx.doi.org/ 10.1111/bcp.12006.

Fox, J., Peters, B., Prakash, M., Arribas, J., Hill, A. and Moecklinghoff., C., 2010. Improvement in vitamin D deficiency following antiretroviral regime change: results from the MONET study. AIDS Research and Human Retroviruses 27, 29-34.

Ginde, A.A., Liu, M.C. and Camargo, C.A., 2009. Demographic differences and trends of vitamin D insufficiency in the US population. Archives of Internal Medicine 169, 626-632.

Giusti, A., Penco, G. and Pioli, G., 2011. Vitamin D deficiency in HIV-infected patients: a systematic review. Nutrition and Dietary Supplements 3, 101-111.

Hammond, E., McKinnon, E., Gendenning, P., Williams, R., Mallal, S. and Phillips, E., 2012. Association between 25-OH vitamin D and insulin is independent of lipoatrophy in HIV. Clinical Endocrinology (Oxford) 76, 201-206.

Haug, C., Müller, P., Aukrust, P. and Froland, S.S., 1994. Subnormal serum concentration of 1,25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. The Journal of Infectious Diseases 169, 889-893.

Havens, P.L., Stephensen, C.B., Hazra, R., Flynn, P.M., Wilson, C.M., Rutledge, B., Bethel, J., Pan, C.G., Woodhouse, L.R., Van Loan, M.D., Liu, N, Lujan-Zilbermann, J, Baker, A, Kapogiannis, B.G. and Mulligan, K., 2012. Vitamin D3 decreases parathyroid hormone in HIV-infected youth being treated with tenofovir: a randomized, placebo-controlled trial. Clinical Infectious Diseases 54, 1013-1025.

Holick, M.F, 2007. Vitamin D deficiency. The New England Journal of Medicine 357, 266-281.

Hyppönen, E., Berry, D., Cortina-Borja, M. and Power, C., 2010. 25-hydroxyvitamin D and pre-clinical alterations in inflammatory and hemostatic markers: a cross sectional analysis in the 1958 British birth cohort. PLoS One 5(5): e10801.

Kim, J.H., Gandhi, V., Psevdos, G., Espinoza, F., Park, J. and Sharp, V., 2012. Evaluation of vitamin D levels among HIV-infected patients in New York city. AIDS Research and Human Retroviruses 28, 235-241.

Kuller, L.H., Tracy, R., Belloso, W., De Wit, S., Drummond, F., Lane, H.C., Ledergerber, B., Lundgren, J., Neuhaus, J., Nixon, D., Paton, N.I. and Neaton, J.D., 2008. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Medicine 5(10):e203.

Kwan, C.K., Eckhardt, B., Baghdadi, J. and Aberg, J.A., 2012. Hyperparathyroidism and complications associated with vitamin D deficiency in HIV-infected adults in New York City, NY. AIDS Research and Human Retroviruses 28, 1025-1032.

Landriscina, M., Altamura, S.A., Roca, L., Gigante, M., Piscazzi, A., Cavalcanti, E., Costantino, E., Barone, C., Cignarelli, M., Gesualdo, L. and Ranieri, E., 2008. Reverse transcriptase inhibitors induce cell differenciation and enhance the immunogenic phenotype in human renal clear-cell carcinoma. International Journal of Cancer 122, 2842-2850.

Lattuada, E., Lanzafame, M., Zoppini, G., Concia, E. and Vento, S., 2009. No influence of nevirapine on vitamin D deficiency in HIV-infected patients. AIDS Research and Human Retroviruses 25, 849-850.

Legeai, C., Vigouroux, C., Souberbielle, J.C., Bouchaud, O., Boufassa, F., Capeau, J., Goujard, C., Meyer, L. and Viard, J.P., 2012. Different associations between 25-hydroxyvitamin D (25OHD) and immunologic/metabolic markers in black and white treatment-naive HIV-infected persons. The ANRS CO09 'COPANA' Cohort Study. 19th Conference on Retroviruses and Opportunistic Infections, Seattle, abstract 883.

Mary-Krause, M., Viard, J.P., Ename-Mkoumazok, B., Bentata, M., Valantin, M.A., Missy, P., Darasteanu, I., Roux, C., Kolta, S., Costagliola, D., Rozenberg, S., 2012. Prevalence of low bone mineral density in HIV-1 infected men and women, and a proposal for screening strategy. Journal of Clinical Densitometry 15, 422-433.

Mehta, S., Giovanucci, E., Mugusi, F.M., Spiegelman, D., Aboud, S., Hertzmark, E., Msamanga, G.I., Hunter, D. and Fawzi, W.W., 2010. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. PLoS One 5(1): e8770.

Mehta, S., Hunter, D.J., Mugusi, F.M., Spiegelman, D., Manji, K.P., Giovanucci, E.L., Hertzmark, E., Msamanga, G.I. and Fawzi, W.W., 2009. Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania. The Journal of Infectious Diseases 200, 1022-1030.

Mehta, S., Mugusi, F.M., Spiegelman, D., Villamor, E., Finkelstein, J.L., Hertzmark, E., Giovannucci, E.L., Msamanga, G.I. and Fawzi, W.W., 2011. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. AIDS Patient Care and STDs 25, 579-585.

Milazzo, L., Mazzali, C., Bestetti, G., Longhi, E., Foschi, A., Viola, A., Vago, T., Galli, M., Parravicini, C. and Antinori, S., 2011. Liver-related factors associated with low vitamin D levels in HIV and HIV/HCV coinfected patients and comparison to general population. Current HIV Research 9, 186-193.

Mithal, A., Wahl, D.A. and Bonjour, J.P., 2009. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International 20, 1807-1820.

Mueller, N.J., Fux, C.A., Ledergerber, B., Elzi, L., Schmid, P., Dang, T., Magenta, L., Calmy, A., Vergopoulos, A. and Bischoff-Ferrari, H.A., 2010. High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients. AIDS 24, 1127-1134.

Ormesher, B., Dhaliwal, S., Nylen, E., Gibert, C., Go, C., Amdur, R. and Benator, D., 2011. Vitamin D deficiency is less common among HIV-infected African-American than in a matched cohort. AIDS 25, 1237-1239.

Pacanowski, J., Valin, N., Guéchot, J., Atbaoui, F., Pillon, A., Kreplak, G., Fonquernie, L., Bottero, J., Girard, P.M. and Meynard, J.L., 2012. Effects of vitamin D3 supplemntation on 25-OH vitamin D and PTH concentrations in 483 HIV+ patients. 19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, USA, abstract 887.

Paul, T.V., Asha, H.S., Thomas, N., Seshadri, M.S., Rupali, P., Abraham, O.C., Pulimood, S.A. and Jose, A., 2010. Hypovitaminosis D and bone mineral density in human immunodeficiency virus-infected men from India, with or without antiretroviral therapy. Endocrine Practice 16, 547-553.

Pocaterra, D., Carenzi, L., Ricci, E., Minisce, D., Schiavini, M., Meraviglia, P., Bevilacqua, M., Zucchi, P., Bonfanti, P. and Rizzardini, G., 2011. Secondary hyperparathyroidism in HIV patients: is there any responsibility of highly antiretroviral therapy? AIDS 25, 1430-1433.

Rockstroh, J., Stoehr, A., Duiculescu, D., Voronin, E., Hill, A., Van Delft, Y. and Marks, S., 2010. Analysis of vitamin D and parathyroid hormone in the SENSE trial: eravirine versus efavirenz in treatment-naïve HIV-1 infected patients. Antiviral Therapy 15, A42-A43.

Rodger, A.J., Fox, Z., Lundgren, J.D., Kuller, L.H., Boesecke, C., Gey, D., Skoutelis, A., Goetz, M.B. and Phillips, A.N., 2009. Activation and coagulation markers are independent predictors of the development of opportunistic disease in patients with HIV infection. The Journal of Infectious Diseases 200, 915-921.

Rosenvinge, M.M., Gedela, K., Copas, A.J., Wilkinson, A., Sheehy, C.A., Bano, G., Hay, P.E., Pakianathan, M.R. and Sadiq, S.T., 2010. Tenofovir-linked hyperparathyroidism is independently associated with the presence of vitamin D deficiency. Jourrnal of Acquired Immune Deficiency Syndromes 54, 496-499.

Ross, A.C., Judd, S., Kumari, M., Hileman, C., Storer, N., Labbato, D., Tangpricha, V. and McComsey, G.A., 2011. Vitamin D is linked to carotid intima-media thickness and immune reconstitution in HIV-positive individuals. Antiviral Therapy 16, 555-563.

Rutstein, R., Downes, A., Zemel, B., Schall, J. and Stallings, V., 2011. Vitamin D status in children and young adult with perinatally acquired HIV infection. Clinical Nutrition 30, 624-628.

Schleithoff, S.S., Zittermann, A., Tenderich, G., Berthold, H.K., Stehle, P. and Koerfer, R., 2006. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled study. The American Journal of Clinical Nutrition 83, 754-759.

Shikuma, C.M., Seto, T., Liang, C.Y., Bennett, K., DeGruttola, V., Gerschenson, M., Stein, J.H., Budoff, M., Hodis, H.N., Delaney, J.A.C., Ogata-Arakaki, D., Pramyothin, P. and Chow, D., 2012. D. Vitamin D levels and markers of arterial dysfunction in HIV. AIDS Research and Human Retroviruses 28, 793-797.

Souberbielle, J.C., Body, J.J., Lappe, J.M., Plebani, M., Shoenfeld, Y., Wang, T.J., Bischoff-Ferrari, H.A., Cavalier, E., Ebeling, P.R., Fardellone, P., Gandini, S., Gruson, D., Guérin, A.P., Heickendorff, L., Hollis, B.W., Ish-Shalom, S., Jean, G., Von Landenberg, P., Largura, A., Olsson, T., Pierrot-Deseilligny, C., Pilz, S., Tincani, A., Valcour, A. and Zittermann, A., 2010. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. Autoimmunity Reviews 9, 709-715.

Stein, E.M., Yin, M.T., McMahon, D.J., Shu, A., Zhang, C.A., Ferris, D.C., Colon, I., Dobkin, J.F., Hammer, S.M. and Shane, E., 2011. Vitamin D deficiency in HIV-infected postmenopausal Hispanic and African American women. Osteoporosis International 22, 477-487.

Szep, Z., Guaraldi, G., Shah, S., Lo Re 3rd,V., Ratcliffe, S.J., Orlando, G., Carli, F., Rossi, R., Rochira, R. and Tebas, P., 2011. Vitamin D deficiency is associated with type 2 diabetes mellitus in HIV infection. AIDS 25, 525-529.

Terrier, B., Carrat, F., Geri, G., Pol, S., Piroth, L., Halfon, P., Poynard, T., Souberbielle, J.C. and Cacoub, P., 2011. Low 25-OH vitamin D levels correlate with severe fibrosis in HIV-HCV co-infected patients with chronic hepatitis. Journal of Hepatology 55, 756-761.

Tseng, A. and Foisy, M., 2012. Important drug-drug interactions in HIV-infected persons on antiretroviral therapy: an update on new interactions between HIV and non-HIV drugs. Current Infectious Disease Reports 14, 67-82.

Van den Bout-Van den Beukel, C., Fievez, L., Michels, M., Sweep, F.C.G.J., Hermus, A.R.M.M., Bosch, M.E.W., Burger, D.M., Bravenboer, B., Koopmans, P.P. and Van der Ven, A.J.A.M., 2008. Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy. AIDS Research and Human Retroviruses 24, 1375-1382.

Van Vonderen, M.G.A., Lips, P., Van Agtmael, M.A., Hassink, E.A.M., Brinkman, K., Geerlings, S.E., Sutinen, J., Ristola, M., Danner, S.A. and Reiss, P., 2009. First line zidovudine/lamivudine/lopinavir/ritonavir leads to greater bone loss compared to nevirapine/lopinavir/ritonavir. AIDS 23, 1367-1376.

Vescini, F., Cozzi-Lepri, A., Borderi, M., Re, M.C., Maggiolo, F., De Luca, A., Cassola, G., Vullo, V., Carosi, G., Antinori, A., Tozzi, V. and D'Arminio-Monforte, A., 2011. Prevalence of hypovitaminosis D and factors associated with vitamin D deficiency and morbidity among HIV-infected patients enrolled on a large Italian cohort Jourrnal of Acquired Immune Deficiency Syndromes 58, 163-172.

Viard, J.P., Souberbielle, J.C., Kirk, O., Reekie, J., Knysz, B., Losso, M., Gatell, J., Pedersen, C., Bogner, J.R., Lundgren, J.D. and Mocroft, A., 2011. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. AIDS 25:1305-1315.

Von Essen, R.M., Kongsbak, M., Schjerling, P., Olgaard, K., Odum, N. and Geisler, C., 2010. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nature Immunology 11, 344-349.

Wang, Z., Lin, Y.S., Zheng, X.E., Senn, T., Hashizume, T., Scian, M., Dickmann, L.J., Nelson, S.D., Baillie, T.A., Hebert, M.F., Blough, D., Davis, C.L. and Thummel, K.E., 2012. An inducible cytochrome P450 3A4-dependent vitamin D catabolic pathway. Molecular Pharmacology 81, 498-509.

Welz, T., Childs, K., Ibrahim, F., Poulton, M., Taylor, C.B., Moniz, C.F. and Post, F.A., 2010. Efavirenz is associated with severe vitamin D deficiency and increased alkalin phosphatase. AIDS 24, 1923-1928.

Wiboonchutikul, S., Sungkanuparph, S., Chailurkit, L.O., Charoenyingwattana, A., Wangsomboonsiri, W., Chantratita, W. and Ongphiphadhanakul, B., 2012. Vitamin D insufficiency and deficiency among HIV-1-infected patients in a tropical setting. Journal of the International Association of Physicians in AIDS Care 11, 305-310.

Wohl, D., Doroana, M., Orkin, C., Pilotto, J.H., Sungkanuparph, S., Yeni, P., Vanveggel, S., Deckx, H. and Boven, K., 2011. Change in vitamin D levels smaller and risk of development of severe vitamin D deficiency lower among HIV-1-infected, treatment-naïve adults receiving TMC278 compared with EFV: 48-week results from the phase III ECHO Trial. 18th Conference on Retroviruses and Opportunistic Infections. Boston, MA, USA, abstract 79LB;

Yang, X., Hashimuze, T., Shhart, M.C., Davis, C.L., Nelson, W.L., Sakaki, T., Kalhorn, T.F., Watkins, P.B., Schuetz, e.g. and Thummel, K.E., 2006. Intestinal and hepatic CYP3A4 catalyze hydroxylation of 1α,25-dihydroxyvitamin D3: implications for drug-induced osteomalacia. Molecular Pharmacology 69, 56-65.

Yin, M.T., Lu, D., Cremers, S., Tien, P.C., Cohen, M.H., Shi, Q., Shane, E., Golub, E.T. and Anastos, K., 2010. Short-term bone loss in HIV-infected premenopausal women Jourrnal of Acquired Immune Deficiency Syndromes 53, 202-208.

Key facts

- The importance of sunlight has been noted throughout the history of tuberculosis treatment. Solar ultraviolet B radiation is the primary source of vitamin D for most people living on earth.
- Tuberculosis is a major global health problem and often coincides with nutritional deficiency.
- Tuberculosis is a complex and multifactorial disease that is influenced by both environmental and genetic factors.
- An association between vitamin D deficiency and tuberculosis has been reported in a number of studies.
- High doses of vitamin D were widely used to treat active tuberculosis before the emergency of antibiotics.

Summary points

- Changes in the environment, such as those caused by tobacco smoking as well as geographic and seasonal factors, may cause diseases that contribute to the development of both vitamin D deficiency and tuberculosis.
- Genetic studies provide an excellent opportunity to link molecular variations, such as polymorphisms, with subtle biological effects. Host genetic factors are important determinants of susceptibility to tuberculosis.
- In a meta-analysis study, low vitamin D levels were associated with higher risk of active tuberculosis.
- Bacillus Calmette Guérin (BCG) vaccination was developed as a vaccine to provide protection against tuberculosis.
- BCG-vaccinated infants are almost 6 times more likely to have sufficient vitamin D concentrations than unvaccinated infants 3 months after BCG vaccination, and this association remained strong even after adjusting for season, ethnic group and sex.
- Clinical and radiological improvements were more evident in children with tuberculosis who were taking vitamin D supplements compared to controls.
- Vitamin D supplementation may be beneficial to individuals with insufficient vitamin D levels.

18. Vitamin D and tuberculosis

K. vinh quốc Lương and L.T.H. Nguyễn
Vietnamese American Medical Research Foundation, 14971 Brookhurst St., Westminster, CA 92683, USA; lng2687765@aol.com

Abstract

Tuberculosis is a major global health problem and often coincides with nutritional deficiency. Tuberculosis is a complex and multifactorial disease that is influenced by both environmental and genetic factors. Changes in the environment, such as those caused by tobacco smoking as well as geographic and seasonal factors, may cause diseases that contribute to the development of both vitamin D deficiency and tuberculosis. Genetic factors, such as mutations (or deletion) in the vitamin D receptor (VDR), major histocompatibility complex regions, chromosome 20, Toll-like receptors, natural resistance-associated macrophage protein 1 and nucleotide-binding oligomerization domain containing proteins 2, contribute to both vitamin D status and tuberculosis. The role that vitamin D plays in tuberculosis has also been demonstrated by its effects on Bacillus Calmette Guérin, vascular endothelial growth factor, matrix metalloproteinases, prostaglandins, reactive oxygen species, and reactive nitrogen intermediates, in addition to its synergistic effects with some anti-tuberculosis medications. Vitamin D plays a definite role in tuberculosis. Vitamin D itself may affect tuberculosis through the VDRs or may influence tuberculosis through indirect effects.

Keywords: vitamin D, calcitriol, tuberculosis, mycobacteria

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_18, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D3$	1,25-dihydroxyvitamin D3
25(OH)D	25-hydroxyvitamin D
BCG	Bacillus Calmette Guérin
DBP	Vitamin D-binding protein
HLA	Human leukocyte antigen
IL-12	Interleukin-12
INF-α	Interferon-alpha
iNOS	Nitric oxide synthase
MDR	Multidrug-resistant
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MyD88	Myeloid differentiation factor 88
NOD2	Nucleotide-binding oligomerization domain containing proteins 2
NRAMP1	Natural resistance-associated macrophage protein 1
PGES	Prostaglandin E synthase
PGs	Prostaglandins
PZA	Pyrazinamide
RNI	Reactive nitrogen intermediates
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
TLR	Toll-like receptor
TNF-γ	Tumor necrosis factor-gamma
UVB	Ultraviolet B
VDR	Vitamin D receptor
VEGF	Vascular endothelial growth factor

18.1 Introduction

Cell-mediated immunity is crucial to the host response to infection with *Mycobacterium tuberculosis* (Lowrie and Andrew, 1988; Rook, 1988a). Upon infection with *M. tuberculosis*, T-cells secrete Th1 cytokines (e.g. INF-γ) and activate macrophages, which enhance the host's ability to kill *M. tuberculosis* and achieve immune protection (Chang-hong *et al.*, 2008). In contrast, Th2 cytokines can reduce macrophage bactericidal activity of macrophages and weaken the immune response to *M. tuberculosis* (Seah *et al.*, 2000). Moreover, the Th1 cytokine INF-γ up-regulates TLR 2/1 induction of 25-hydroxyvitamin D3-1α-hydroxylase, leading to the conversion of 25(OH)D to its active metabolite $1,25(OH)_2D3$. In contrast, Th2 cytokines induce the catabolism of 25(OH)D to the inactive metabolite $24,25(OH)_2D$ (Edfeldt *et al.*, 2010). In a mouse model of tuberculosis, Th1 cells accumulation and the activation of myeloid cells were noted in *M. tuberculosis*-infected lungs, but a decreased accumulation of Th1 cells on day 21 was also noted (Kang *et al.*, 2011). The production of Th2 cytokines increases as a model of

active pulmonary tuberculosis progresses and worsens, but Th2 cytokine levels decrease when the disease is cured (North, 1988). Patients with MDR display impaired Th1 responses (Geffner *et al.*, 2009) and suppressed Th1 and Th2 cell activation (Tan *et al.*, 2012). On the other hand, calcitriol has some paradoxical effects on host resistance to infection. Treating tuberculosis with calcitriol is associated with decreased Th1 mediated immunity but increased bactericidal activity and tuberculosis clearance in infected human macrophages (Cantorna *et al.*, 2008). Vitamin D supplements influence the development of a Th2 response in murine allergic airway disease (Matheu *et al.*, 2003). Vitamin D3 attenuates Th2 responses to *Aspergillus fumigatus* (Kreindler *et al.*, 2010). Calcitriol also has anti-inflammatory effects on a Th2-dependent asthma model (Topilski *et al.*, 2004). Furthermore, malnutrition is known to suppress immunity (Good, 1981), and a possible link between vitamin D deficiency and impaired host defense against *M. tuberculosis* has been suggested (Davies, 1985; Lương and Nguyễn, 2011). In the present paper, we will review the role of vitamin D in tuberculosis.

18.2 Risk factors for the development of both vitamin D deficiency and tuberculosis

Tuberculosis is a complex and multifactorial disease that is influenced by both environmental and genetic factors. It has been reported that vitamin D deficiency and tuberculosis share many of the same risk factors, including both environmental and genetic risk factors.

18.2.1 Environmental factors

Changes in the environment, such as those caused by tobacco smoking as well as geographic and seasonal factors, may cause diseases that contribute to the development of both vitamin D deficiency and tuberculosis.

Tobacco smoking

Smoking is a major health hazard that exhibits side effects on many organs. Tobacco users are at risk for low bone mineral density, decreased intestinal calcium absorption, and low 25(OH)D plasma levels (Jorde *et al.*, 2005; Krall and Dawson-Hughes, 1999; Lorentzon *et al.*, 2007; Need *et al.*, 2002; Supería *et al.*, 2006). The newborns of smoking mothers have significantly lower anthropometric measurements and serum 25(OH)D levels (Diaz-Gómez *et al.*, 2007). In addition, the VDR *TaqI TT* polymorphism was moderately associated with both the presence and the progression of periodontitis in smokers, while no association was detected in non-smoking individuals (Nibali *et al.*, 2008). On the other hand, both passive and active exposure to tobacco smoke were associated with susceptibility to tuberculosis (Basu *et al.*, 2011; Jee *et al.*, 2009; Leung *et al.*, 2010; Tachfouti *et al.*, 2011; Wang *et al.*, 2009), but this risk was substantially reduced when individuals quit smoking (Awaisu *et al.*, 2011; Wen *et al.*, 2010). Evidence from meta-analysis studies suggested an association between smoking and tuberculosis (Bates *et al.*, 2007; Slama *et al.*, 2007). In addition, the association between active exposure to tobacco smoke and tuberculin

activity has been reported in many studies (Anderson *et al.*, 1997; Nisar *et al.*, 1993). Tuberculosis patients who are smokers are more likely to have more cavity lesions (Abal *et al.*, 2005; Trifunović *et al.*, 2009), and an increased the risk of morbidity and mortality were observed in tuberculosis patients who smoked (Jiang *et al.*, 2009; Racil *et al.*, 2010). These findings suggested that tobacco smoking may contribute to the development of vitamin D deficiency and tuberculosis.

Geographic factors

It has been observed that there is a progressive decrease in solar UVB radiation exposure with each 10-degree deviation from the equator (Diffey, 1991). Solar UVB radiation is the primary source of vitamin D for most people living on Earth. As latitude increases, vitamin D-producing UV radiation decreases dramatically, potentially inhibiting vitamin D synthesis in humans (Kimlin *et al.*, 2007). In Boston, USA (42.2° North latitude), human skin does not produce any previtamin D3 when exposed to sunlight on cloudless days from November through February. In Edmonton, Canada (52° North latitude), this ineffective period extends from October through March. In areas further south (between latitudes 34° North and 18° North), sunlight effectively photo-converts dehydrocholesterol to previtamin D3 in the middle of winter (Webb *et al.*, 1988). Similarly, a case-control study in Asian-Indians immigrants living in South London has shown a significant trend of an increased risk of tuberculosis in these immigrants compared with the indigenous UK population (Strachan *et al.*, 1995). Vitamin D deficiency has been suggested as a factor contributing to the increased rates of tuberculosis reactivation among those emigrating from tropical areas (high UVB exposure) to temperate climates (low UVB exposure) (Rook, 1988b). The molecular analysis of isolates has shown that immigrants may acquire tuberculosis after arrival in the host country and that transmission between foreign-born and autochthonous individuals may occur in both directions (Garzelli and Rindi, 2012).

Seasonal factors

Significant seasonal vitamin D variations were reported in both southern and northern latitudes (Oliveri *et al.*, 1993; Stryd *et al.*, 1979). Barger-Lux and Heany (2002) confirmed and quantified the relatively large seasonal fluctuations in 25(OH)D levels that are associated with sun exposure in outdoor workers. The median serum 25(OH)D levels of the workers decreased from 122 nmol/l in late summer to 74 nmol/l in late winter. Similarly, a seasonal pattern has been detected in tuberculosis. In Russia, the number of hospitalizations related to tuberculosis was significantly higher in cold months than in warm months (Atun *et al.*, 2005). In the Voronezh region, the highest incidence of tuberculosis was registered in winter months (Shilova and Glumnaia, 2004). In South Africa, there is an increased transmission of childhood tuberculosis in the autumn and winter (Schaaf *et al.*, 1996). An inverse relationship between seasonal variations in vitamin D status and tuberculosis diagnosis was observed in Cape Town, South Africa (Martineau *et al.*, 2011). In addition, seasonal variations in tuberculosis rates and a prevalence of tuberculosis in the winter months were reported in South Western Cameroon (Ane-Anyangwe *et al.*, 2006). In the systemic review of peer-reviewed studies, the risk of transmission of *M. tuberculosis* appears to be the greatest during the winter months (Fares, 2011). Moreover, the seasonality of pulmonary

tuberculosis was also reported in migrant workers entering Kuwait (Akhtar and Mohammad, 2008). The migrants have a very high proportion of tuberculosis and arrive mainly in the winter months, while migrants from Sri Lanka and other low burden countries have a low proportion of tuberculosis and arrive mainly in the summer.

18.2.2 Genetic factors

Genetic studies provide an excellent opportunity to link molecular variations such as polymorphisms to subtle biological effects. Host genetic factors are important determinants of tuberculosis susceptibility.

Chromosome 20

A genomic DNA clone for 1,25$(OH)_2$D3 24-hydroxylase has been isolated from a human chromosome 20 library (Chen and DeLuca, 1995). In a genome-wide association study, a variant at the *20q* locus was identified as a risk factor for vitamin D insufficiency (Wang *et al.*, 2010a). A region of chromosome 20 has been linked to tuberculosis susceptibility in Thais (Mahasirimongkol *et al.*, 2009). However, the co-localization of these loci on the same chromosome does not necessarily that they are related.

Human leukocyte antigen

The HLA system is the major histocompatibility complex in humans. The primary function of the HLA system is to regulate the immune response. This system is involved in conferring susceptibility to or protection from mycobacterial disease in animal and humans. It has been shown that tuberculosis susceptibility can be influenced by MHC haplotype. Knockout mice with defined HLA class II deficiencies were infected with *M. bovis* BCG, whereas controls were able to inhibit bacterial growth and resist infection (Ladel *et al.*, 1995). A number of studies have reported an association between HLA class II alleles and tuberculosis, but these associations are not consistent across different populations. HLA studies have revealed that the following HLA alleles may be associated with a higher risk of tuberculosis; such as, *DQA1*0101* in the Iranian population (Amirzargar *et al.*, 2004), *DQB1*0301-0304* in the South African population (53), *DQB1*05* in the South African and Polish populations (Lombard *et al.*, 2006; Dubaniewicz *et al.*, 2003), *DQB1*0501* in the American Indian and Mexican populations (Teran-Escandon *et al.*, 1999), *DQB1*0502* in the Thai population (Vejbaesya *et al.*, 2002), *DQB1*0503* in the Cambodian population (Goldfeld *et al.*, 1998), *DQB1*0601* in the Korean and Asian Indian populations (Kim *et al.*, 2005; Ravikumar *et al.*, 1999), *DQB1*0602/3* in the South African population (Lombard *et al.*, 2006), *DRB1*07* in the Iranian population (Dubaniewicz *et al.*, 2003), *DRB1*0803* in the Korean population (Kim *et al.*, 2005), *DRB1*1101-1121* in the South African population (Lombard *et al.*, 2006), *DRB1*1101* and *DRB1*12* in the Indonesian population with recurrent tuberculosis (Yuliwulandari *et al.*, 2010), *DRB1*13* in the Polish population (Dubaniewicz *et al.*, 2000), *DRB1*1302* in the South African population (Lombard *et al.*, 2006), *DRB1*14* in the Iranian (Mahmoudzardeh-Niknam and Fadavi, 2003), Tyvian (Matrashkin *et al.*, 2002), and Portuguese

populations (Duarte *et al.*, 2011), *DRB1*1501* in the Asian Indian (Mehra *et al.*, 1995; Ravikumar *et al.*, 1999) and Chinese populations (Shi *et al.*, 2011), *DR16* in the Chinese population (Liu *et al.*, 2004a), and *DRB1*16* in the Polish population (Dubaniewicz *et al.*, 2000). In contrast, other alleles such as the following may be associated with a lower risk of tuberculosis: *DQB1*02* and *DRB1*13* in the Polish population (Dubaniewicz *et al.*, 2000; Lombard *et al.*, 2006), *DQA1*0301* and *0501* in the Iranian population (Amirzargar *et al.*, 2004), *DRB1*1202* in the absence of *B*1802* in the Indonesian population (Ravikumar *et al.*, 1999), and *DRB1*13* in the Chinese population (Liu *et al.*, 2004a). On the other hand, calcitriol is known to stimulate phagocytosis but suppresses MHC class II antigen expression in human mononuclear phagocytes (Tokuda and Levy, 1996; Tokuda *et al.*, 1992), thereby preventing antigen-specific T cell proliferation. In addition, calcitriol exerts effects that oppose the effect that IL-4 has on MHC class-II antigen expression in human monocytes (Xu *et al.*, 1993). Calcitriol specifically modulates human monocyte phenotype and function, altering HLA-DR antigen expression and antigen presentation, while leaving lytic function intact (Rigby *et al.*, 1990). Calcitriol also decreases interferon-gamma-induced HLA-DR antigen expression on normal and transformed human keratinocytes (Tamaki *et al.*, 1990-1991; Tone *et al.*, 1991) and reduces the levels of HLA-DR mRNA in cultured epithelial tumor cell lines (Tone *et al.*, 1993). In addition, 1α-calcidol significantly modulated the expression of HLA-DR in human peripheral blood monocytes (Scherberich *et al.*, 2005). The relationship between allelic variations in HLA and tuberculosis summarized in Table 18.1.

Vitamin D receptors

The levels of VDR protein were significantly decreased in tuberculosis (Selvaraj *et al.*, 2009). The VDR genes have also been suggested to influence the cell-mediated immune response to the *M. tuberculosis* antigen in tuberculosis patients (Selvaraj *et al.*, 2000a). Several polymorphisms that may affect the clinical outcome and severity of the disease were found in the *VDR* gene. *VDR* polymorphisms have wide ranging roles in innate immunity and are associated with tuberculosis. Four common polymorphisms (*TaqI*, *ApaI*, *BsmI* and *FokI*) in the *VDR* gene were studied in clinically diagnosed tuberculosis. There is no evidence that *TaqI* is associated with tuberculosis in Tibetan and Cambodian populations (Chen *et al.*, 2006a; Delgado *et al.*, 2002). However, it has been reported that the *TaqI tt* genotype occurred more frequently in female tuberculosis (Selvaraj *et al.*, 2000b) and was found to be associated a with higher risk of smear positive tuberculosis in Asian Indians (Sharma *et al.*, 2011). However, this genotype might be associated with resistance to tuberculosis in Africans (Bellamy *et al.*, 1999). The *Tt* genotype increased the probability of culture conversion during tuberculosis treatment (Roth *et al.*, 2004) and *T*-containing genotypes predict a faster response to tuberculosis chemotherapy (Babb *et al.*, 2007). In Paraguayans, the *TaqI t* allele protects against active tuberculosis disease but not infection; individuals with a *TT* genotype are 42 times less likely to mount a delayed-type hypersensitivity response (Wilbur *et al.*, 2007). It seems that the *ApaI Aa* and *aa* genotypes indicate a high of smear positive disease in the general population and in Muslim populations of central India, respectively (Sharma *et al.*, 2011). *ApaI AA* genotypes were predictive of a faster response to tuberculosis treatment (Babb *et al.*, 2007). There is a significantly higher frequency of *BsmI Bb* in male tuberculosis (Selvaraj *et al.*, 2003). The *bb* genotype conferred a significant risk for smear positive and MDR

Table 18.1. The relationship between allelic variations in HLA and tuberculosis.

HLA		Ethnicity
Higher risk of tuberculosis		
DQA1	*0101*	Iranian
DQB1	*0301-0304*	South African
	05	South African and Polish
	0501	American Indian and Mexican
	0502	Thai
	0503	Cambodian population
	0601	Korean and Asian Indian
	0602/3	South African
DRB1	*07*	Iranian
	0803	Korean
	1101-1121	South African
	1101 and 12	Indonesian
	13	Polish
	1302	South African
	14	Iranian, Tyvian and Portuguese
	1501	Asian Indian and Chinese
	16	Polish
Lower risk for tuberculosis		
DQA1	*0301 and 0501*	Iranian
DQB1	*02*	Polish (in the absence of *B*1802*)
DQB1	*13*	Polish and Chinese
Role of vitamin D		Suppress MHC class II antigen expression
		Reduce the levels of HLA-DR mRNA

tuberculosis in the Muslims of central India (Sharma *et al.*, 2011). In another study, the *BsmI BB* genotype was reported to increase susceptibility to tuberculosis, while the *bb* genotype decreased susceptibility to tuberculosis in the Turkish (Ates *et al.*, 2011). It was reported that the *FokI ff* genotype occurred more frequently in Tibetans and Gujarati Asians in West London (Chen *et al.*, 2006b; Wilkinson *et al.*, 2000). Asian Indians with *Ff* and *ff* genotypes were at a high risk of developing MDR and smear positive tuberculosis disease (Sharma *et al.*, 2011). The *FokI F* allele protects Paraguayans from tuberculosis. *FF* genotypes are 17 times more likely to test positive for exposure to tuberculosis but are no more likely to have ever been diagnosed with active tuberculosis (Wilbur *et al.*, 2007). *FokI* polymorphisms in the *VDR* gene are also associated with susceptibility to extrapulmonary tuberculosis (Motsinger-Reif *et al.*, 2010) and spinal tuberculosis in the Chinese Han population (Zhang *et al.*, 2010). There is no evidence of a *FokI* association with tuberculosis in the Tibetan population (Chen *et al.*, 2006b). In a family-based

study, the transmission-disequilibrium test analysis showed a significantly greater association between the SNP combinations *FokI-BsmI-ApaI-TaqI* or *FokI-ApaI* and tuberculosis than between individual SNPs and tuberculosis (Bornman *et al.*, 2004). A study on host susceptibility confirmed the association of *BsmI* (*BB*+*bb*) SNPs in the *VDR* and SNPs in the *TNF-γ* genes with susceptibility to tuberculosis in Iranians (Merza *et al.*, 2009). Moreover, the rate of tuberculosis among Canadian Aboriginals was higher than that among Canadian-born non-Aboriginals (27.4 vs. 2 cases/100,000 individuals); the Canadian Aboriginal populations exhibits a significantly higher frequency of SNPs that are associated with lower expression of VDR, INF-α, and TNF-α, and higher monocyte chemoattractant protein-1 production compared to a Caucasian cohort (Larcombe *et al.*, 2008). In meta-analysis studies, polymorphisms at *FokI* loci (*VDR-ff*) showed a statistically significant association with tuberculosis susceptibility; however, a variant in *TaqI* was not associated with tuberculosis (Zhao *et al.*, 2009). However, Gao *et al.* (2010) found that *VDR* polymorphisms (*FokI*, *TaqI*, *ApaI* and *BsmI*) are associated with tuberculosis in Asians and that none of these polymorphisms were significantly related to tuberculosis in Africans or South Americans. The *Cdx-2 GG* genotype, which is a SNP in *VDR*, is associated with protection from tuberculosis (Selvaraj *et al.*, 2008). In a case-control study, the combined *VDR* genotypes *AbfT* and *AabbFfTT* were statistically significant factors that protected Iranian against tuberculosis (Marashian *et al.*, 2010). The *VDR* haplotype *FbAT* also significantly protected South Africans from tuberculosis (Lombard *et al.*, 2006). Recent studies have identified methylation variable positions in the 3' *CpG* island that distinguish ethnicity and tuberculosis status (Andraos *et al.*, 2011). Moreover, a *Gc* gene variant of the DBP is correlated with decreased circulating levels of 25(OH)D, 1,25$(OH)_2$D3 and DBP (Lauridsen *et al.*, 2005; Abbas *et al.*, 2008) and was strongly associated with susceptibility to active tuberculosis in Gujarati Asians who are living in London (Martineau *et al.*, 2010). Finally, studies from different populations have determined that *VDR* polymorphisms may have differential susceptibility or resistance to tuberculosis. The relationship between allelic variations in *VDR* and tuberculosis is summarized in Table 18.2.

Human toll-like receptors

TLRs are a group of glycoproteins that function as surface trans-membrane receptors and are involved in innate immune responses to exogenous pathogenic microorganisms. The production of IL-12, TNF-α, and NO are induced soon after the innate recognition of mycobacteria through TLRs (Underhill *et al.*, 1999). TLR2 expression was up-regulated on monocytes obtained from tuberculous pleural fluid; TLR2 and TLR4 expression were also enhanced on INF-γ secreting $CD4^+$ T cells (Prabha *et al.*, 2008). MyD88-deficient mice, which lack a critical component of TLR signaling, failed to control acute and chronic *M. avium* growth and succumbed to infection 9-14 weeks post-infection. Infected $TLR2^{-/-}$ mice also showed increased susceptibility but displayed longer survival times and a lower bacterial burden than $MyD88^{-/-}$ mice. Infected $TLR4^{-/-}$ mice were indistinguishable from wild-type animals. The histopathological examination of $MyD88^{-/-}$ mice revealed the massive destruction of lung tissue, which was not present in the wild-type, $TLR2^{-/-}$, or $TLR4^{-/-}$ mice (Feng *et al.*, 2003). $TLR2^{-/-}$ mice, however, succumbed to *M. tuberculosis* infection (Drennan *et al.*, 2004) due to compromised IL-23 expression in response to *M. tuberculosis* (Teixeira-Coelho *et al.*, 2011). The role played by TLR4 in susceptibility to *M. tuberculosis* varies

Table 18.2. The relationship between allelic variations in VDR and tuberculosis.

Gene	Link to tuberculosis
VDR polymorphism	
TaqI	No association with tuberculosis in Tibetan and Cambodian populations.
tt genotype	Occurs more frequently in female tuberculosis patients.
	Associated with high risk of smear positive tuberculosis in Asian Indians.
	Associated with resistance to tuberculosis in Africans.
t genotype	Protects against active tuberculosis in Paraguayans.
Tt genotype	Increased probability of culture conversion during tuberculosis treatment.
T-containing genotype	Predictive of a faster response to tuberculosis chemotherapy.
TT genotype	Forty-two times less likely to mount a delayed-type hypersensitivity response in Paraguayans.
ApaI	
Aa genotype	Is at high risk of tuberculosis in general population of central India.
aa genotype	Is at high risk of tuberculosis in Muslims of central India.
BmsI	
Bb genotype	Occurs more frequently in male tuberculosis patients.
	Increased frequency susceptibility to tuberculosis in Turkish populations.
bb genotype	Risk of smear positive and multiple drug resistant (MDR) tuberculosis in Muslims of central India.
	Decreased frequency susceptibility to tuberculosis in Turkish population.
FokI	Associated with extrapulmonary tuberculosis and spinal tuberculosis.
ff genotype	Occurs more frequently in tuberculosis Tibetan and Guajarati Asians in West London.
Ff and *ff* genotypes	High risk of MDR and smear positive tuberculosis in Asian Indians.
F genotype	Protects Paraguayans from tuberculosis.
FF genotype	17 times more likely to test positive for exposure to tuberculosis, but no more likely to have ever been diagnosed with active tuberculosis.
Combination of SNPs (*FokI-ApaI* and *FokI-BsmI-ApaI-TaqI*)	Higher risk of tuberculosis than an individual SNP.
Association of SNPs in *BsmI* (*BB+bb*) and *TNF-γ* genes	Susceptibility to tuberculosis in Iranians.
Cdx-2 GG genotype	Associated with protection against tuberculosis.
Combined *AbfT* and *AabbFffTT*	Protects Iranians against tuberculosis.
Haplotype *FbAT*	Protects South Africans from tuberculosis.
DBP alleles	
Gc gene variant	Decreased levels of 25(OH)D, 1,25(OH)D and DBP.
	Associated with susceptibility to active tuberculosis among Gujarati Asians living in London.

between different studies (Branger *et al.*, 2004; Shim *et al.*, 2003). Cooperation between TLR2 and TLR4 mediated signaling is involved in *M. tuberculosis*-induced macrophage death (Sánchez *et al.*, 2010). TLR9 is involved in the control of *M. avium* infection but is not related to the induction of Th1 responses (Carvalho *et al.*, 2011). Changes in the structure of TLR signaling molecules might be associated with susceptibility to various infectious diseases. Variants of *TLR1* and *TLR6* were associated with an altered-immune response to BCG vaccination in South African infants (Randhawa *et al.*, 2011). *TLR2* polymorphisms are associated with susceptibility to tuberculosis in Tunisians (Ben-Ali *et al.*, 2004), Koreans (Yim *et al.*, 2006), Vietnamese (Thuong *et al.*, 2007), Taiwanese (Chen *et al.*, 2010), and Turkish children (Dalgic *et al.*, 2011). The *S/M TLR2* genotype may increase susceptibility to tuberculosis in Han Chinese, but the *S/L* genotype may act as a negative risk factor (Xue *et al.*, 2010a). Variants in *TLR2* and *9* might also play important roles in determining tuberculosis susceptibility in Caucasians, African-Americans, and West Africans (Velez *et al.*, 2010). *TLR4* polymorphisms influence the susceptibility and severity of tuberculosis in Asian Indians (Najmi *et al.*, 2010), HIV-infected patients in Tanzania (Ferwerda *et al.*, 2007), and the Caucasian population in the Mediterranean (Pulido *et al.*, 2010), but are not limked to the southeastern Chinese (Xue *et al.*, 2010b) and Gambian populations (Newport *et al.*, 2004). *TLR6* polymorphisms were associated with altered IL-6 levels in response to *M. tuberculosis* and BCG (Shey *et al.*, 2010). *TLR8* polymorphisms are associated with tuberculosis susceptibility in Indonesian males (Davila *et al.*, 2008), and male Turkish children (Dalgic *et al.*, 2011). However, the allele and genotype frequencies of the various *TLR* genes are not risk factors for tuberculosis in South Indian (Selvaraj *et al.*, 2010). On the other hand, calcitriol suppresses the expression of TLR2 and TLR4 protein and mRNA in human monocytes and triggers hyporesponsiveness to pathogen-associated molecular patterns (Sadeghi *et al.*, 2006). Calcitriol has also been shown to down-regulate intracellular TLR2, TLR4 and TLR 9 expression in human monocytes (Dickie *et al.*, 2010). TLR activation results in the expression of the VDR and 1α-vitamin D hydroxylase in human monocytes, leading to the induction of the antimicrobial peptide cathelicidin and the killing of intracellular *M. tuberculosis* (Liu *et al.*, 2006). Calcitriol can cause the vitamin D-induced expression of cathelicidin in bronchial epithelial cells (Yim *et al.*, 2007) and may enhance the production of cathelicidin LL-37 (Rivas-Santiago *et al.*, 2008). Poor vitamin D intake may increase susceptibility to *M. tuberculosis* infection by inefficiently supporting the induction of *cathelicidin mRNA* expression in monocytes (Liu *et al.*, 2006; 2007). The addition of a VDR antagonist inhibited the induction of *cathelicidin* mRNA by more than 80%; consequently, the protein expression of this antimicrobial agent was reduced by approximately 70% (Liu *et al.*, 2006). Furthermore, the knockdown of cathelicidin in primary monocytes results in the loss of TLR-mediated antimicrobial activity against intracellular mycobacteria (Liu *et al.*, 2009). Upon *M. tuberculosis* stimulation, calcitriol modulates cytokine production towards an anti-inflammatory profile by decreasing the expression of TLR2 and TLR4, while increasing cathelicidin production (Khoo *et al.*, 2011). Calcitriol also induces autophagy in human monocytes via cathelicidin and leads to the co-localization of mycobacterial phagosomes and autophagosomes in human macrophages in a cathelicidin-dependent manner (Yuk *et al.*, 2009). These findings suggested that vitamin D down-regulates the pro-inflammatory cytokine responses to *M tuberculosis* through pattern recognition receptors while inducing the production of antimicrobial cathelicidin.

The natural resistance-associated macrophage protein 1

Nramp1 (now renamed SLC11α1-solute carrier family 11a member 1) plays a critical role in macrophage defenses against intracellular pathogens. The expression of *Nramp1* in pathogen-containing phagosomes is associated with enhanced fusion to lysosomes, increased phagosomal acidification, and greater bactericidal activity. The *NRAMP1* gene has been shown to regulate the concentration of divalent cations in the phagosomes of macrophage (Forbes and Gros, 2001). The antibacterial role of *NRAMP1* could be a result of the extrusion of protons and divalent metal ions from the phagosomal lumen toward the cytoplasm (Jabado *et al.*, 2000). Polymorphonuclear leukocytes are the major site of *NRAMP1* expression, followed to a lesser degree by monocytes (Cellier *et al.*, 1997). The *Nramp1* gene has been identified in inbred mice as a factor for host defense against some mycobacteria species (Vidal *et al.*, 1995). In mice, *Nramp1* polymorphisms cause susceptibility to mycobacterial infections and affect intracellular bacterial replication by modulating phagosomal pH (Hackam *et al.*, 1998). The bovine *NRAMP1* gene, however, does not determine resistance and susceptibility to infection with *M. bovis* in cattle (Barthel *et al.*, 2000). *NRAMP1* polymorphisms may have an effect on *Bacillus* growth and the outcomes of pulmonary tuberculosis but do not have an effect on susceptibility to *M. tuberculosis* infection (Zhang *et al.*, 2005). In humans, several *NRAMP1* polymorphisms seem to be associated with genetic susceptibility to tuberculosis in Caucasian (Ma *et al.*, 2002), Chinese (Liu *et al.*, 2004b), Tibetan (Chen *et al.*, 2009), Indonesian (Surabaya) (Nugraha and Anggraini, 2011), Korean (Ryu *et al.*, 2000), West African (Cervino *et al.*, 2000), Cambodian population (Delgado *et al.*, 2002), and Greek population (Stagas *et al.*, 2011). Other *NRAMP1* variants, however, were not a risk factor for tuberculosis in the Taiwanese (Liaw *et al.*, 2002), Japanese (Abe *et al.*, 2003), Indonesian (South Sulawesi) (Hatta *et al.*, 2010), Thai (Vejbaesya *et al.*, 2007), Moroccan (Baghdali *et al.*, 2003), and Mexican populations (Niño-Moreno *et al.*, 2007). Significant associations between tuberculosis risk and widely studied *NRAMP1* polymorphisms were observed in a systemic review and meta-analysis study (Li *et al.*, 2011). On the other hand, calcitriol is known to stimulate phagocytosis (Tokuda and Levy, 1996). Calcitriol affects *NRAMP1* transcription and protein expression in maturing phagocytes (Roig *et al.*, 2002). Moreover, Sp1 and CCAAT enhancer-binding protein are reported to regulate the expression of the *NRAMP1* gene in myeloid cells (Richer *et al.*, 2008). CCAAT enhancer-binding protein is a molecular target of calcitriol in breast cancer (Dhawan *et al.*, 2009) and is induced by calcitriol in Th17 cells (Chang *et al.*, 2010). The mVDR promoter is controlled by Sp1 sites (Jehan and DeLuca, 2000) and functions as the transactivation component of the VDR/Sp1 complex during gene expression (Chen *et al.*, 2006a).

The nucleotide-binding oligomerization domain containing protein 2

The NOD receptor family is a diverse set of twenty two innate immune receptors that are involved in the cytoplasmic detection of bacteria and the activation of inflammatory cascades. NOD2 has been shown to have a role in the recognition of mycobacteria (Ferwerda *et al.*, 2005) and is an intracellular receptor that regulates the host response to *M. tuberculosis* and *M. bovis* and the response to BCG infection in human macrophages (Brooks *et al.*,2011). NOD2-deficient mice had a higher mycobacterial burden in the lung six months after infection and succumbed to

infection sooner than wild-type controls did (Divangahi *et al.*, 2008). The important role that NOD2 plays in the recognition of *M. tuberculosis* was demonstrated in the mononuclear cells of individuals who are homozygous for a *NOD2* mutation. These cells showed an 80% lower cytokine response after stimulation with *M. tuberculosis* (Ferwerda *et al.*, 2005). Nonsynonymous *NOD2* polymorphisms have been associated with active tuberculosis in African Americans (Austin *et al.*, 2008), but not in the African Gambian population (Stockton *et al.*, 2004). Similarly, caspase-recruitment domain-containing protein 15, which encodes the NOD2 protein, is not a major susceptibility gene for tuberculosis in the South African population (Möller *et al.*, 2007). On the other hand, calcitriol strongly stimulates NOD2 expression in differentiated human THP-1 macrophage-like cells, primary human monocytes, and keratinocytes (Wang *et al.*, 2010a,b). Calcitriol also robustly stimulates the expression of pattern recognition receptor *NOD2/CARD15IDB1* in primary human monocyte and epithelial cells (Wang *et al.*, 2010b).

18.3 The role of vitamin D in tuberculosis

An association between vitamin D deficiency and tuberculosis has been reported in a number of studies. Low vitamin D levels were associated with a five-fold increased risk for progression to tuberculosis (Talat *et al.*, 2010). A vegetarian diet, which is associated with a low plasma vitamin D level, is an independent risk factor for active tuberculosis among Asian immigrants living in South London (Strachan *et al.*, 1995). Tuberculosis patients also have significantly lower mean concentrations of serum 25(OH)D compared with healthy subjects (Davies *et al.*, 1985; Sita-Lunsden *et al.*, 2009). A correlation between serum levels of vitamin D and risk for latent tuberculosis has been noted among African immigrants living in Australia (Gibney *et al.*, 2008). Patients with chronic renal failure are also at an increased risk for developing tuberculosis (Cuss *et al.*, 1986), because certain uremic toxins can suppress $1,25(OH)_2D3$ synthesis and its biological activity (Hsu and Patel, 1997). In a meta-analysis study, low vitamin D levels were associated with a higher risk of active tuberculosis (Nnoaham and Clark, 2008).

The BCG vaccine was developed to provide protection against tuberculosis. BCG-vaccinated infants are almost 6 times more likely to have sufficient vitamin D concentrations than unvaccinated infants 3 months after BCG vaccination, and this association remains strong even after adjusting for season, ethnic group and sex (Lalor *et al.*, 2011). Among the vaccinated group, there was also a strong inverse correlation between the IFN-γ response to *M. tuberculosis* PPD and vitamin D concentration; infants with higher vitamin D concentrations had lower IFN-γ responses. Similarly, tuberculosis in cattle usually presents with a rapid transient increase in serum calcitriol within the first two weeks following infection (Rhodes *et al.*, 2003). $1,25(OH)_2D3$-positive mononuclear cells were later identified in all of the tuberculous granulomas. During tuberculosis infection, alveolar macrophage-produced calcitriol plays a beneficial role by limiting inflammation-mediated tissue injury, potentiating NO production by stimulated monocytes/macrophages, inhibiting INF-γ production by stimulated $CD4^+$ cells, and suppressing the growth of *M. tuberculosis* (Ametaj *et al.*, 1996; Rockett *et al.*, 1998). In persons with positive tuberculin test results, calciferol is able to suppress the tuberculin reaction (Huff, 1963). A single dose of vitamin D has been reported to enhance immunity to mycobacteria (Martineau *et al.*, 2007). The

VDR genes have also been suggested to influence the cell-mediated immune response to the *M. tuberculosis* antigen in tuberculosis patients (Selvaraj *et al.*, 2000a).

High doses of vitamin D were widely used to treat active tuberculosis in the preantibiotic era. The importance of sunlight has been noted throughout the history of tuberculosis treatment. In 1854, Herman Brehmer, a Silesian botany student who was suffering from tuberculosis, traveled to the Himalayan mountains to pursue his botanical interests and cured his tuberculosis (Warren, 2006). Vitamin D was used to treat tuberculosis of the bone and caused some improvement (Pattison, 1929). UVB exposure, however, is sufficient to double the circulating 25(OH)D levels (from 11.23 ng/ml to 20.39 ng/ml), but no significant changes have been observed in antimycobacterial immunity (Yesudian *et al.*, 2008), which might be attributed to low vitamin D levels in patients with tuberculosis. Clinical and radiological improvements were more evident in *M. tuberculosis*-infected children taking additional vitamin D than controls (Morcos *et al.*, 1998). Serum 25(OH)D concentrations increased during the first two months of tuberculosis treatment (Tostmann *et al.*, 2010). Vitamin D is known to suppress the intracellular growth of *M. tuberculosis in vitro* (Rockett *et al.*, 1998). Calcitriol was shown to inhibit the multiplication of virulent tubercle bacilli in cultured human macrophages *in vivo* (Crowle *et al.*, 1987). Calciferol was reported to dissolve cavities in tuberculosis patients (Brincourt, 1969). In a random trial, the use of multivitamin supplements, including vitamin D, reduced HIV-infected tuberculosis patient mortality by 50% (Range *et al.*, 2006). Moreover, vitamin D supplementation resulted in more rapid sputum clearance of acid-fast bacilli and radiological improvement in Indonesian tuberculosis patients (Nursyam *et al.*, 2006). The correction of vitamin D deficiency and anti-tuberculosis treatments have also resulted in clinical and microbiological improvements in a refractory drug-susceptible tuberculosis patient (Yamshchikov *et al.*, 2009). In another trial, however, vitamin D did not improve the clinical outcomes of tuberculosis patients, demonstrating no overall effect on the mortality of tuberculosis patients (Wejse *et al.*, 2009). This finding may have been due to a suboptimal dosage.

MMPs are proteolytic enzymes that are responsible for extracellular matrix remodeling and the regulation of leukocyte migration through the extracellular matrix, which is an important step in inflammatory processes and infectious diseases. MMPs are produced by many cell types including lymphocytes, granulocytes, astrocytes and activated macrophages. The expression of *MMPs-1*, *-2*, *-3*, *-7*, and *-9* was increased in human astrocytes that were stimulated by conditioned medium from *M. tuberculosis*-infected monocytes (Harris *et al.*, 2007). MMP-mediated proteolysis of the mycobacterial heat shock protein 65 was reported to contribute to the complex immunomodulatory interplay during the course of tuberculosis infection (Shiryaev *et al.*, 2011). In tuberculosis patients, the circulating level of MMP-9 is correlated with disease severity (Hrabec *et al.*, 2002) and granuloma formation (Sheen *et al.*, 2009). In addition, infection with *M. tuberculosis* has been shown to decrease the expression of TIMPs-1, -2 and -3 in peripheral blood mononuclear cells and human epithelial cells (Brew *et al.*, 2000). Patients with an *MMP-1*(-1607G) polymorphism are more vulnerable to extensive lung fibrosis one year after anti-tuberculosis treatment (Wang *et al.*, 2010c), and the *1G* genotypes of *MMP-1* polymorphisms were associated with a greater risk of developing tracheobronchial stenosis in the Taiwanese (Kuo *et*

al., 2008). In addition, the mouse MMP-1 ortholog is not expressed in the lung and mice infected with *M. tuberculosis* do not develop tissue destruction equivalent to that in humans. In MMP-1 transgenic mice, however, *M. tuberculosis* infection increased *MMP-1* expression, resulting in alveolar destruction in lung granulomas and significantly greater collagen breakdown (Elkington *et al.*, 2011). On the other hand, VDR knockout mice had increased influx of inflammatory cells, phospho-acetylation of nuclear factor-kappaB associated with increased pro-inflammatory cells, and up-regulation of MMP-2, MMP-9, and MMP-12 in the lung (Sundar *et al.*, 2011). The *VDR TaqI* polymorphism is associated with the decreased production of tissue metalloproteinase inhibitor-1, which is a natural inhibitor of MMP-9 (Timms *et al.*, 2002). Calcitriol modulates tissue MMP expression under experimental conditions (Dean *et al.*, 1996), down-regulates MMP-9 levels in keratinocytes, and may attenuate the deleterious effects caused by the excessive TNF-α-induced proteolytic activity associated with cutaneous inflammation (Bahar-Shany *et al.*, 2010). Calcitriol inhibits both basal and *Staphylococcus*-stimulated production of MMP-9 in human blood monocytes and alveolar macrophages (Lacraz *et al.*, 1994). Moreover, a vitamin D analog was also reported to reduce the expression of MMP-2, MMP-9, VEGF and parathyroid hormone-related peptide in Lewis lung carcinoma cells (Nakagawa *et al.*, 2005). Furthermore, calcitriol significantly attenuated *M. tuberculosis*-induced increases in the expression of MMP-7 and MMP-10, and suppressed the secretion of MMP-7 by *M. tuberculosis*-infected PBMCs. MMP-9 gene expression, secretion and activity were significantly inhibited, irrespective of infection status (Coussens *et al.*, 2009). In another study, calcitriol also suppressed the production of MMPs (MMP-7 and MMP-9) and enhanced the level of TMP-1 in tuberculosis patients (Anand and Selvaraj, 2009). These studies suggested that calcitriol may play an important role in the pathological process of tuberculosis by down-regulating the levels of MMPs and regulating the levels of TIMPs.

Angiogenesis is a complex process involving the coordinated steps of endothelial cell activation, proliferation, migration, tube formation and capillary sprouting, and requires the participation of intracellular signaling pathways. VEGF is a key mediator of angiogenesis. The vascular changes associated with angiogenesis usually occur in cancer, but have also been reported in inflammatory process diseases. VEGF gene expression was reported to be up-regulated in macrophages infected with *M. tuberculosis* (Ragno *et al.*, 2001). The serum levels of VEGF were significantly higher in patients with active pulmonary tuberculosis than in patients with old tuberculosis and acute bronchitis; the decrease in serum VEGF titer paralleled the clinical improvement of pulmonary tuberculosis (Abe *et al.*, 2001; Matsuyama *et al.*, 2000; Alatas *et al.*, 2004). *Mycobacteria* induce pleural mesothelial permeability and release VEGF from mesothelial cells by down-regulating β-catenin expression (Mohammed *et al.*, 2003; Seiscento *et al.*, 2010). The serum and cerebrospinal fluid levels of VEGF were significantly higher in tuberculous meningitis than in other meningitis; the decrease in cerebrospinal fluid VEGF titer paralleled the clinical improvement of tuberculous meningitis (Matsuyama *et al.*, 2001). In these studies, the immuno-histochemical staining of autopsied brains demonstrated the presence of VEGF in the mononuclear cells of the dense fibroconnective tissue in both the subarachnoid space and surrounding the vasculitis lesions. VEGF is a known a marker of disease activity in neurotuberculosis (Husain *et al.*, 2008). In contrast, calcitriol was reported to inhibit angiogenesis *in vitro* and *in vivo* (Mantell *et al.*, 2000).

Calcitriol significantly inhibits VEGF-induced endothelial cell spouting and elongation in a dose-dependent manner and decreases the production of VEGF (Gruber *et al.*, 2008). Calcitriol is a potent inhibitor of retinal neovascularization in mouse model of oxygen-induced ischemic retinopathy (Albert *et al.*, 2007). Vitamin D and its analog also reduce the expression of VEGF in various cancer cell lines (Ben-Shoshan *et al.*, 2007; Nakagawa *et al.*, 2005). Moreover, *DBP-macrophage activating factor* was reported to inhibit angiogenesis and tumor growth in mice (Kisker *et al.*, 2003) and inhibited the VEGF signaling by decreasing the VEGF-mediated phosphorylation of VEGF-2 and ERK1/2, a downstream target of the VEGF signaling cascade (Kalkunte *et al.*, 2005).

PGs play a role in the inflammatory process. Cyclooxygenase participates in the conversion of arachidonic acid into PGs. PGs regulate the cell-mediated immune response and have a role in the progression of pulmonary tuberculosis. VEGF stimulates the expression of cyclooxygenase-2 and PGES in response to cell activation (Sakurai *et al.*, 2004). During the chronic phase of experimental murine tuberculosis, an inhibitor of PG synthesis enhanced Th1 cytokine levels, decreased Th2 cytokines levels, increased the expression of iNOS and reduced pulmonary inflammatory, fibrosis and mycobacteria load (Hernández-Pando *et al.*, 2006). *In vitro* and *in vivo* studies using $PGES^{-/-}$ mice, which cannot produce PGE_2, showed a significantly higher mycobacterial burden in these mice than control mice. These findings suggested that PGE_2 plays a critical role in the inhibition of *M. tuberculosis* replication (Chen *et al.*, 2008). In tuberculosis patients, the production of PGE was increased in both lungs, but the hyperproduction of $PGF_{2\alpha}$ occurred in the impaired lung (Kaminskaia *et al.*, 1991). The spontaneous development of tuberculosis in guinea pigs was accompanied by phasic changes in PG. There was an increase in PGs during a swift progression of inflammatory and necrotic changes, with an abundance of PGF being noted; however, the PG content in the lung tissue normalized after tuberculosis treatment (Kaminskaia *et al.*, 1990). On the other hand, calcitriol has been reported to regulate the expression of several key genes involved in the PG pathway, causing a decrease in PG synthesis (Moreno *et al.*, 2005). Calcitriol and its analogs have been shown to selectively inhibit the activity of cyclooxygenase-2 (Apama *et al.*, 2008). These findings suggested that vitamin D plays a role in modulating the inflammatory process in tuberculosis.

ROS are produced by activated phagocytes as a part of their microbicidal activities. ROS are capable of damaging the host tissue through lipid peroxidation. The end product of lipid peroxidation, MDA, serves as a marker of cellular damage. The serum ROS levels in active lung tuberculosis and sequel lung tuberculosis cases were significantly higher than those of the control group (Ceylan *et al.*, 2005). The levels of lipid peroxidation end products, 9,11-linoleic acid, and free radical activity were higher in the active pulmonary tuberculosis cases than the inactive pulmonary tuberculosis cases (Jack *et al.*, 1994). The high levels of free radicals in active pulmonary cases decreased to normal at the end of treatment (Makinskii *et al.*, 2002). In another study, MDA and lipid peroxidation products in sputum smear-positive patients with advanced pulmonary tuberculosis and sputum smear-negative patients with small radiographic changes were significantly higher than those of a healthy control groups (Kwiatkowska *et al.*, 1999). The elevated exhalation of hydrogen peroxide was also reported in patients with pulmonary

tuberculosis (Kwiatkowska *et al.*, 2007). Decreased SOD activity was noted in the disseminated stage of military and infiltrative tuberculosis. SOD activity is known to increase when there is no dissemination (Safarian and Karapetian, 1990). Similarly, calcitriol has been reported to exert a receptor-mediated effect on the secretion of hydrogen peroxide by human monocytes (Cohen *et al.*, 1986). Human monocytes in culture gradually lose their capacity to produce superoxide when stimulated. The addition of calcitriol, lipopolysaccharide or lipoteichoic acid restored the ability of stimulated monocytes to produce superoxide and increased their oxidative capacity compared with unstimulated monocytes (Levy an Malech, 1991). Calcitriol can also protect nonmalignant prostate cells from oxidative stress-induced cell death by eliminating ROS-induced cellular injuries (Bao *et al.*, 2008). Vitamin D metabolites and vitamin D analogs were reported to induce lipoxygenase mRNA expression, lipoxygenase activity and ROS in a human bone cell line (Somjen *et al.*, 2011). Vitamin D can also reduce the extent of lipid peroxidation and induce SOD activity in the hepatic anti-oxidant system in rats (Sardar *et al.*, 1996). These findings suggested that vitamin D modulates oxidative stress in tuberculosis.

Cytokine-activated macrophages produce RNI to combat pathogens. *In vitro*, the expression of RNI at physiological concentrations may prevent *M. tuberculosis* from proliferating, but the removal of RNI allows mycobacteria to proliferate (Firmani and Riley, 2002). In most murine models, iNOS is essential for the killing of intracellular mycobacteria (Chan *et al.*, 1992), $iNOS^{-/-}$ mice proved highly susceptible to *M. tuberculosis*, which exhibited faster replication in the lungs of these mice than in the lungs of hosts deficient in other genes (MacMicking *et al.*, 1997), and iNOS inhibitors also render similar deleterious effects on tuberculosis infection in mice, as assessed by mortality, bacterial burden, and pathological tissue damage (Chan *et al.*, 1995). These findings confirmed the importance of RNI in resistance against *M. tuberculosis*. In granulomatous diseases, such as tuberculosis, the activation of macrophage 1α-hydroxylase results in an increase in $1,25(OH)_2D3$, which inhibits iNOS expression and reduces NO production by LPS-stimulated macrophages (Chang *et al.*, 2004). This calcitriol production by macrophages may provide protection against the oxidative injuries caused by the NO burst. Calcitriol is known to inhibit LPS-induced immune activation in human endothelial cells (Equils *et al.*, 2005). Calcitriol enhances the intracellular glutathione pools and significantly reduces the nitrite production that is induced the by LPS (Garcion *et al.*, 1999). In human macrophage-like cells, calcitriol induces iNOS and suppresses the growth of *M. tuberculosis* (Rockett *et al.*, 1998). Finally, vitamin D affects the RNI to combat tuberculosis.

PZA is an important medication for tuberculosis, and plays a key role in shortening tuberculosis treatment from 9-12 months to 6 months. UV light enhances the activity of PZA against *M. tuberculosis in vitro* (Wade and Zhang, 2006). These authors suggested that UV light can generate free radicals that cause damage to macromolecules – such as DNA – and can also affect membrane integrity. UVB radiation, however, was reported to induce calcitriol from 7-dehydrocholesterol in human keratocytes (Lehmann *et al.*, 2000; Vantieghem *et al.*, 2006). Calcitriol synergizes with PZA to kill tuberculous bacilli in cultured human macrophages (Crowle *et al.*, 1989). Chloroquine has been used to control hypercalcemia in granulomatous patients (O'Leary *et al.*, 1986), and chloroquine therapy has been associated with a significant reduction in the

serum levels of $1,25(OH)_2D3$ and urinary calcium, perhaps via inhibition of the conversion of 25(OH)D to $1,25(OH)_2D3$. A combination of three drugs (chloroquine, $1,25(OH)_2D3$, and PZA) kill tuberculous bacilli faster than the combination of 1,25(OH)D and PZA in cultured human macrophages (Crowle and May, 1990). Ketoconazole has been reported to decrease the serum levels of ionized calcium and $1,25(OH)_2D3$ in tuberculosis-associated hypercalcemia (Saggese *et al.*, 1993) and has also been shown to act against *M. tuberculosis* both *in vitro* and in a mouse model (Byrne *et al.*, 2007). Ketoconazol, however, was reported to synergize with calcitriol by inhibiting 24-hydroxylase and increasing the activity of calcitriol in human skin and prostate cancer cells (Kang *et al.*, 1997; Peehl *et al.*, 2002).

18.4 Conclusion

The relationship between vitamin D and tuberculosis has been discussed. Vitamin D clearly has a beneficial role in some patients with tuberculosis. Genetic studies have provided the opportunity to determine which proteins link vitamin D to tuberculosis pathology. Vitamin D also exerts its effect on tuberculosis through non-genomic mechanisms. It is necessary to check the vitamin D status of patients with tuberculosis. Calcitriol is best used for tuberculosis because it is the active form of vitamin the D3 metabolite, has receptors in the lung, and inhibits inflammatory cytokine expression.

References

Abal, A., Jayakrishnan, B., Parwer, S., El Shamy, A., Abahussain, E. and Sharma, P., 2005. Effect of cigarette smoking on sputum smear conversion in adults with active pulmonary tuberculosis. Respir Med. 99, 415-420.

Abbas, S., Linseisen, J., Slanger, T., Kropp, S., Mutschelknauss, E., Flesch-Janys, D. and Chang-Claude, J., 2008. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. Cancer Epidemiol Biomarkers Prev. 17, 13339-1343.

Abe, Y., Nakamura, M., Oshika, Y., Hatanaka, H., Tokunaga, T., Ohkubo, Y., Hashizume, T., Suzuki, K. and Fujino, T., 2001. Serum levels of vascular endothelial growth factor and cavity formation in active pulmonary tuberculosis. Respiration. 68, 496-500.

Abe, T., Iinuma, Y., Ando, M., Yokoyama, T., Yamamoto, T., Nakashima, K., Takagi, N., Baba, H., Hasegawa, Y. and Shimokata, K., 2003. NRAMP1 polymorphisms, susceptibility and clinical features of tuberculosis. J Infect. 46, 215-220.

Akhtar, S. and Mohammad, H., 2008. Seasonality in pulmonary tuberculosis among migrant workers entering Kuwait. BMC Infect Dis. 8, 3.

Alatas, F., Alatas, Ö., Metinas, M., Özarslan, A., Erginel, S. and Yildirim, H., 2004. Vascular endothelial growth factor levels in active pulmonary tuberculosis. Chest. 125, 2156-2159.

Albert, D., Scheef, E., Wang, S., Mehraein, F., Darjatmoko, S., Sorenson, C. and Sheibani, N., 2007. Calcitriol is a potent inhibitor of retinal neovascularization. Invest Ophthalmol Vis Sci. 48, 2327-2334.

Ametaj, B., Beitz, D., Reihardt, T. and Nonnecke, B., 1996. 1,25-dihydroxyvitamin D3 inhibits secretion of interferon-gamma by mitogen- and antigen-stimulated bovine mononuclear leukocytes. Vet Immunol Imnunopathol. 52, 77-90.

Amirzargar, A., Yalda, A., Hajabolbaghi, M., Khosravi, F., Jabbari, H., Rezaei, N., Niknam, M., Ansari, B., Moradi, B. and Nikbin, B., 2004. The association of HLA-DRB, DQB1 alleles and haplotype frequency in Iranian patients with pulmonary tuberculosis. Int J Tuberc Lung Dis. 8, 1017-1021.

Anand, S. and Selvaraj, P., 2009. Effect of 1,25 dihydroxyvitamin D3 on matrix metalloproteinases MMP-7, MMP-9 and the inhibitor TIMP-1 in pulmonary tuberculosis. 133, 126-131.

Anderson, R., Sy, F., Thompson, S. and Addy, C., 1997. Cigarette smoking and tuberculin skin test conversion among incarcerated adults. Am J Prev Med. 13, 175-181.

Ane-Anyangwe, I., Akenji, T., Mbacham, W., Penlap, V. and Titanji, V., 2006. Seasonal variation and prevalence of tuberculosis among health seekers in the South Western Camaroon. East Afr Med J. 83, 588-595.

Andraos, C., Koorsen, G., Knight, J. and Bornman, L., 2011. Vitamin D receptor gene methylation is associated with ethnicity, tuberculosis, and TaqI polymorphism. Hum Immunol. 72, 262-268.

Aparna, R., Subhashini, J., Roy, K., Reddy, G., Robinson, M., Uskokovic, M., Venkateswara Reddy, G. and Reddanna, P., 2008. Selective inhibition of cyclooxygenase-2 (COX-2) by 1alpha,25-dihydroxy16-ene-yne-vitamin D3, a less calcemic vitamin D analog. J Cell Biochem. 104, 1832-1842.

Ates, O., Dolek, B., Dalyan, L., Musellim, B., Ongen, G. and Topal-Sarikaya, A., 2011. The association between BsmI variant of vitamin D receptor gene and susceptibility to tuberculosis. Mol Biol Rep. 38, 2633-2636.

Atun, R., Samyshkin, Y., Drobniewski, F., Kuznetsov, S., Fedorin, I. and Coker, R., 2005. Seasonal variation and hospital utilization for tuberculosis in Russia: hospitals as social care institutions. Eur J Pub Health. 15, 350-354.

Austin, C., Ma, X. and Graviss, F., 2008. Common nonsynonymous polymorphisms in the NOD2 gene are associated with resistance or susceptibility to tuberculosis disease in African Americans. J Infect Dis. 197, 1713-1716.

Awaisu, A., Haniki Nik Mohamed, M., Noordin, N., Muttalif, A., Aziz, N., Syed Sulaiman, S. and Mahayiddin, A., 2011. The SCIDOTS project: evidence of benefits of an integrated tobacco cessation intervention in tuberculosis care on treatment outcomes. Substance Abuse Treatment, Prevention, and Policy. 6, 26.

Babb, C., van der Merwe, L., Beyers, N., Pheiffer, C., Walzl, G., Duncan, K., van Helden, P. and Hoal, E., 2007. Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. Tuberculosis (Edinb). 87, 295-302.

El Baghdadi, J., Remus, N., Benslimane, A., El Annaz, H., Chentoufi, M., Abel, L. and Schurr, E., 2003. Variants of the human NRAMP1 gene and susceptibility to tuberculosis in Morocco. Int J Tuberc Lung Dis. 7, 599-602.

Bahar-shany, K., Ravid, A. and Koren, R., 2010. Upregulation of MMP-production by TNFalpha in keratinocytes and its attenuation by vitamin D. J Cell Physiol. 222, 729-737.

Bao, B., Ting, H., Hsu, J. and Lee, Y., 2008. Protective role of 1α-dihydroxyvitamin D3 against oxidative stress in nonmalignant human prostate epithelial cells. Int J Cancer. 122, 2699-2706.

Barger-Lux, M. and Heany, R., 2002. Effects of above average summer sun exposure on serum 25-hydroxyvitamin D and calcium absorption. J Clin Endocrinol Metab. 87, 4952-4956.

Barthel, R., Piedrahita, J., McMurray, D., Payeur, J., Baca, D., Suárez Güemes, F., Perumaalla, V., Ficht, T., Templeton, J. and Adams, L., 2000. Pathologic findings and association of *Mycobacterium bovis* infection with the bovine NRAMP1 gene in cattle from herds with naturally occurring tuberculosis. Am J Vet Res. 61, 1140-1144.

Basu, S., Stuckler, D., Bitton, A. and Glantz, S., 2011. Projected effects of tobacco smoking on worldwide tuberculosis control: mathematical modeling analysis. BMJ. 343, d5506.

Bates, M., Khalakdina, A., Pai, M., Chang, L., Lessa, F. and Smith, K., 2007. Risk of tuberculosis from exposure to tobacco smoke. Arch Intern Med. 167, 335-342.

Bellamy, R., Ruwende, C. and Corrah, T., 1999. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor. J Infect Dis. 179, 721-724.

Ben-Ali, M., Barbouche, M., Bousnina, S., Chabbou, A. and Dellagi, K., 2004. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol. 11:625-626.

Ben-Shoshan, M., Amir, S., Dang, D., Dang, L., Weisman, Y. and Mabjeesh, N., 2007. 1α,25-dihydroxyvitamin D3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth pathway in human cancer cells. Mol Cancer Ther. 6, 1433-1439.

Bornman, L., Campbell, S., Fielding, K., Bah, B., Sillah, J., Gustafson, P., Manneh, K., Lisse, I., Allen, A., Sirugo, G., Sylla, A., Aaby, P., McAdam, K., Bah-Sow, O., Bennett, S., Lienhardt, C. and Hill, A., 2004. Vitamin D receptor polymorphisms and susceptibility to tuberculosis in West Africa: a case-control and family study. J Infect Dis. 190, 1631-1641.

Branger, J., Leemans, J., Florquin, S., Weijer, S., Speelman, P. and van der Poll, T., 2004. Toll-like receptor 4 plays a protective role in pulmonary tuberculosis in mice. Int Immunol. 16, 509-516.

Brew, K., Dinakarpandian, D. and Nagase, H., 2000. Tissue inhibitors of metalloproteinase: evolution, structure and function. Biochem Biophys Acta. 2, 267-283.

Brincourt, J., 1969. Liquefying effect on suppuration of an oral dose of calciferol. Presse Med. 77, 467-470.

Brooks, M., Rajaram, M., Azad, A., Amer, A., Valdivia-Arenas, M., Park, J., Núñez, G. and Schlesinger, L., 2011. NOD2 controls the nature of the inflammatory response and subsequent fate of *Mycobacterium tuberculosis* and *M. bovis* BCG in human macrophages. Cell Microbiol. 13, 402-418.

Byrne, S., Denkin, S., Gu, P., Nuermberger, E. and Zhang, Y., 2007. Activity of ketoconazole against *Mycobacterium tuberculosis in vitro* and in the mouse model. J Med Microbiol. 56, 1047-1051.

Cantorna, M., Yu, S. and Bruce, D., 2008. The paradoxical effects of vitamin D on type 1 mediated immunity. Mol Aspects Med. 29, 369-375.

Carvalho, N., Oliveira, F., Durães, F., de Almeida, L., Flórido, M., Prata, L., Caliari, M., Appelberg, R. and Oliveira, S., 2011. Toll-like receptor 9 is required for full host resistance to *Mycobacterium avium* infection but plays no role in induction of Th1 responses. 79, 1638-1646.

Cellier, M., Shustik, C., Dalton, W., Rich, E., Hu, J., Malo, D., Schurr, E. and Gros, P., 1997. Expression of the human NRAMP1 gene in professional primary phagocytes: studies in blood cells and in HL-60 promyelocytic leukemia. J Leukoc Biol. 61, 96-105.

Cervino, A., Lakiss, S., Sow, O. and Hill, A., 2000. Allelic association between the NRAMP1 gene and susceptibility to tuberculosis in Guinea-Conarkry. Ann Hum Genet. 64, 507-512.

Ceylan, E., Gülsün, A., Gencer, M. and Aksoy, N., 2005. A new parameter in the detection of tuberculosis activity: reactive oxygen metabolites. Respiration. 72, 156-159.

Chan, J., Xing, Y., Magliozzo, R. and Bloom, R., 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermadiates produced by activated murine macrophages. J Exp Med. 175, 1351-1359.

Chan, J., Tanaka, K., Carroll, D., Flynn, J. and Bloom, B., 1995. Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis*. Infect Immun. 63, 736-740.

Chang, J., Kuo, M., Kuo, H., Hwang, S., Tsai, J., Chen, H. and Lai, Y., 2004. 1 alpha,25-hydroxyvitamin D3 regulates inducible nitric oxide synthase messenger RNA expression and nitric oxide release in macrophage-like RAW 264.7 cells. J Lab Clin Med. 143, 14-22.

Chang, S., Chung, Y. and Dong, C., 2010. Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. J Biol Chem. 285, 38751-38755.

Chang-hong, S., Xiao-wu, W., Hai, Z., Ting-fen, Z., Li-Mei, W. and Zhi-kai, X., 2008. Immune responses and protective efficacy of the gene vaccine expressing Ag85B and ESAT6 fusion protein from *Mycobacterium tuberculosis*. DNA Cell Biol. 27, 199-207.

Chen, K. and DeLuca, H., 1995. Cloning of the human 1 alpha,25-hydroxyvitamin D3 24-hydroxylase gene promoter and identification of two vitamin D-responsive elements. Biochim Biophys Acta. 1263, 1-9.

Chen, H., Chen, J., Huang, Y., Chang, H. and Hung, W., 2006a. Function role of VDR in the activation of p27Kip1 by the VDR/Sp1 complex. J Cell Biochem. 98, 1450-1456.

Chen, X., Feng, Y., Ma, Y., Zhang, Z., Li, C., Wen, F., Tang, X. and Su, Z., 2006b. Study on the association of two polymorphisms of the vitamin D receptor (VDR) gene with the susceptibility to pulmonary tuberculosis (PTB) in Chinese Tibetans. Sichuan Da Xue Xue Bao Yi Xue Ban. 37, 847-851. [Article in Chinese]

Chen, M., Divangahi, M., Gan, H., Shin, D., Hong, S., Lee, D., Serhan, C., Behar, S. and Remold, H., 2008. Lipid mediators in innate immunity against tuberculosis: opposing roles of PGE_2 and LXA_4 in the induction of macrophage death. J Exp Med. 205, 2791-2801.

Chen, X., Feng, Y., Ma, Y., Zhang, Z., Li, C., Wen, F., Tang, X. and Su, Z., 2009. A study on the haplotype of the solute carrier family 11 memmber 1 gene in Tibertan patients with pulmonary tuberculosis in China. Zhonghua Jie He He Hu Xi Za Zhi. 32, 360-364. [Article in Chinese]

Chen, Y., Hsiao, C., Chen, C., Chin, C., Liu, S., Wu, C., Eng, H., Chao, T., Tsen, C., Wang, Y. and Lin, M. 2010., Toll-like receptor 2 gene polymorphisms, pulmonary tuberculosis, and natural killer cell counts. BMC Med Genet. 11: 17.

Cohen, M., Mesler, D., Snipes, R. and Gray, T., 1986. 1α-dihydroxyvitamin D3 activates secretion of hydrogen peroxide by human monocytes. J Immunol. 136, 1049-1053.

Coussens, A., Timms, P., Boucher, B., Venton, T,, Ashcroft, A., Skolimowska, K., Newton, S., Wilkinson, K., Davidson, R, Griffiths, C, Wilkinson, R. and Martineau, A., 2009. 1α,25-dihydroxyvitamin D3 inhibits matrix metalloproteinases induced by *Mycobacterium tuberculosis* infection. Immunol. 127, 539-548.

Crowle, A., Ross, E. and May, M., 1987. Inhibition by $1,25(OH)_2$-vitamin D3 of the multiplication of virulent tubercle bacilli in cultured human macrophages. Infect Immun. 55:2945-2950.

Crowle, A., Salfinger, M. and May, M., 1989. $1,25(OH)_2$-vitamin D3 synergizes with pyrazinamide to kill tubercle bacilli in cultured human macrophages. Am Rev Respir Dis. 139, 549-552.

Crowle, A. and May, M., 1990. Inhibition of tubercle bacilli in cultured human macrophages by chloroquine used alone and in combination with streptomycin, isoniazide, pyrazinamide, and two metabolites of vitamin D3. Antimicrob Agents Chemother. 34, 2217-2222.

Cuss, F., Carmichael, D., Linington, A. and Hulme, B., 1986. Tuberculosis in renal failure: a high incidence in patients born in the Third World. Clin Nephrol. 25, 129-133.

Dalgic, N., Tekin, D., Kayaalti, Z., Cakir, E., Soylemezoglu, T. and Sancar, M., 2011. Relationship between toll-like receptor 8 gene polymorphisms and pediatric pulmonary tuberculosis. Dis Markers. 31, 33-38.

Davies, P., 1985. A possible link between vitamin D deficiency and impaired host defense to *Mycobacterium tuberculosis*. Tuberc. 66, 301-306.

Davies, P., Brown, R. and Woodhead, 1985. Serum concentrations of vitamin D metabolites in untreated tuberculosis. Thorax. 40, 167-190.

Davila, S., Hibberd, M., Hari Dass, R., Wong, H., Sahiratmadja, E., Bonnard, C., Alisjahbana, B., Szeszko, J., Balabanova, Y., Drobniewski, F., van Crevel, R., van de Vosse, E., Nejentsev, S., Ottenhoff, T. and Seielstad, M., 2008. Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. PLoS Genetics. 4, e1000218.

Dean, D., Schwartz, Z., Schmitz, J., Muniz, O., Lu, Y., Calderon, F., Howell, D. and Boyan, B., 1996. Vitamin D regulation of metalloproteinase activity in matrix vesicles. Connect Tissue Res. 35, 331-336.

Delgado, J., Baena, A., Thim, S. and Goldfeld, A., 2002. Ethnic-specific genetic associations with pulmonary tuberculosis. J Infect Dis. 186, 1463-1468.

Dhawan, P., Wieder. R. and Christakos, S., 2009. CCAAT enhancer-binding protein alpha is a molecular target of 1,25-dihydroxyvitamin D3 in MCF-7 breast cancer cells. J Biol Chem. 284, 3086-95.

Díaz-Gómez, N., Mendoza, C., González-González, N., Barroso, F., Jiménez-Sosa, A., Domenech, E., Clemente, I., Barrios, Y. and Moya, M., 2007. Maternal smoking and the vitamin D-parathyroid hormone system during the perinatal period. J Pediatr. 151, 618-623.

Dickie, L., Church, L., Coulthard, L., Mathews, R., Emery, P. and McDermott, M., 2010. Vitamin D3 downregulates intracellular toll-like receptor 9 expression and toll-like receptor 9-induced IL-6 production in human monocytes. Rheumatol. 48, 1466-1471.

Diffey, B., 1991. Solar ultraviolet radiation effects on biologic systems. Phys Med Biol. 36, 299-328.

Divangahi, M., Mostowy, S., Coulombe, F., Kozak, R., Guillot, L., Veyrier, F., Kobayashi, K., Flavell, R., Gros, P. and Behr, M., 2008. NOD2-deficient mice have impaired resistance to *Mycobacterium tuberculosis* infection through defective innate and adaptive immunity. J Immunol. 181, 7157-7165.

Drennan, M., Nicolle, D., Quesniaux, V., Jacobs, M., Allie, N., Mpagi, J., Frémond, C., Wagner, H., Kirschning, C. and Ryffel, B., 2004. Toll-like receptor 2-deficient mice succumb to *Mycobacterium tuberculosis* infection. Am J Path. 164, 49-57.

Duarte, R., Carvalho, C., Pereira, C., Bettencourt, A., Carvalho, A., Villar, M., Domingos, A., Barros, H., Marques, J., Pinho Costa, P., Mendonça, D. and Martins, B., 2011. HLA class II alleles as markers of tuberculosis susceptibility and resistance. Rev Port Pneumol. 17, 15-19.

Dubaniewicz, A., Lewko, B., Moszkowska, G., Zamorska, B. and Stepinski, J., 2000. Molecular subtypes of the HLA-DR antigens in pulmonary tuberculosis. Int J Infect Dis. 4, 129-133.

Dubaniewicz, A., Moszkowska, G., Szczerkowska, Z. and Hoppe, A., 2003. Analysis of DQB1 allele frequencies in pulmonary tuberculosis: preliminary report. Thorax. 58, 890-891.

Edfeldt, K., Liu, P., Chun, R., Fabri, M., Schenk, M., Wheelwright, M., Keegan, C., Krutzik, S., Adams, J., Hewison, M. and Modlin, R., 2010. T-cell cytokines differentially control human monocyte antimicrobial responses by regulating vitamin D metabolism. Proc Natl Acad Sci USA. 107, 22593-22598.

Elkington, P., Shiomi, T., Breen, R., Nuttall, R., Ugarte-Gil, C., Walker, N., Saraiva, L., Pedersen, B., Mauri, F., Lipman, M., Edwards, D., Robertson, B., D'Armiento, J. and Friedland, J., 2011. MMP-1 drives immunopathology in human tuberculosis and transgenic mice. J Clin Invest. 121, 1827-1833.

Equils, O., Naiki, Y., Shapiro, A., Michelsen, K., Lu, D., Adams, J. and Jordan, S., 2005. 1,25-hydroxyvitamin D3 inhibits lipopolysaccharide-induced immune activation in human endothelial cells. Clin Exp Immunol. 143, 58-64.

Fares, A., 2011. Seasonality of Tuberculosis. J Glob Infect Dis. 3, 46-55.

Feng, C., Scanga, C., Collazo-Custodio, C., Cheever, A., Hieny, S., Caspar, P. and Sher, A., 2003. Mice lacking myeloid differentiation factor 88 display profound defects in host resistance and immune responses to Mycobacterium infection not exhibited by Toll-like receptor 2 (TLR2)- and TLR4-deficient animals. J Immunol. 171, 4758-4764.

Ferwerda, G., Girardin, S., Kullberg, B., Le Bourhis, L., de Jong, D., Langenberg, D., van Crevel, R., Adema, G., Ottenhoff, T., Van der Meer, J. and Netea, M., 2005. NOD2 and Toll-like receptors are nonredundant recognition systems of *Mycobacterium tuberculosis*. PLos Pathog. 1, e34.

Ferwerda, B., Kibiki, G., Netea, M., Dolmans, W. and van der Ven, A., 2007. The toll-like receptor 4 Asp299Gly variant and tuberculosis susceptibility in HIV-infected patients in Tanzania. AIDS. 21, 1375-1377.

Firmani, M and Riley, L., 2002. Reactive nitrogen intermediates have a bacteriostatic effect on *Mycobacterium tuberculosis in vitro*. J Clin Microbiol. 40, 3162-3166.

Forbes, J. and Gros, P., 2001. Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. Trends Microbiol. 9, 397-403.

Garzelli, C. and Rindi, L., 2012. Molecular epidemiological approaches to study the epidemiology of tuberculosis in low-incidence settings receiving immigrants. Infect Genet Evol. 12, 610-618.

Gao, L., Tao, Y., Zhang, L. and Jin, Q., 2010. Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. Int J Tuberc Lung Dis. 14, 15-23.

Garcion, E., Sindji, L., Leblondel, G., Brachet, P. and Darcy, F., 1999. 1,25-hydroxyvitamin D3 regulates the synthesis of γ-glutamyl transpeptidase and glutathione levels in rat primary astrocytes. J Neurochem. 73, 859-866.

Geffner, L., Yokobori, N., Basile, J., Schierloh, P., Balboa, L., Romero, M., Ritacco, V., Vescovo, M., González Montaner, P., Lopez, B., Barrera, L., Alemán, M., Abatte, E., Sasiain, M. and de la Barrera, S., 2009. Patients with multidrug-resistant tuberculosis display impaired Th1 responses and enhanced regulatory T-cell levels in response to an outbreak of multidrug-resistant *Mycobacterium tuberculosis M* and *Ra* strains. Infect Immun. 77, 5025-5034.

Gibney, K., MacGregor, L., Leder, K., Torresi, J., Marshall, C., Ebeling, P. and Biggs, B., 2008. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin Infect Dis. 46, 443-446.

Goldfeld, A., Delgado, J., Thim, S., Bozon, M., Uglialoro, A,, Turbay, D., Cohen, C. and Yunis, E., 1998. Association of an HLA-DQ allele with clinical tuberculosis. JAMA. 279, 226-228.

Good, R., 1981. Nutrition and Immunity. J Clin Immunol. 1, 3-11.

Gruber, H., Hoelscher, G., Ingram, J., Chow, Y., Loffler, B. and Hanley Jr., E., 2008. 1α,25-dihydroxyvitamin D3 inhibits proliferation and decreases production of monocyte chemoattractant protein-1, thrombopoietin, VEGF, and angiogenin by human annulus cells *in vitro*. Spine. 33, 755-765.

Hackam, D., Rotstein, O., Zhang, W., Gruenheid, S., Gros, P. and Grinstein, S., 1998. Host resistance to intracellular infection: mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phogosomal acidification. J Exp Med. 188, 351-364.

Harris, J., Nuttall, R., Elkington, P., Green, J., Horncastle, D., Graeber, M., Edwards, D. and Friedland. J., 2007. Monocyte-astrocyte networks regulate matrix metalloproteinase gene expression and secretion in central nervous system tuberculosis *in vitro* and *in vivo*. J Immunol. 178, 1199-1207.

Hatta, M., Ratnawati, Tanaka, M., Ito, J., Shirakawa, T. and Kawabata, M., 2010. NRAMP1/SLC11A1 gene polymorphisms and host susceptibility to *Mycobacterium tuberculosis* and *M. leprae* in South Sulawesi, Indonesia. Southeast Asian J Trop Med Public Health. 41, 386-394.

Hernández-Pando, R., Orozco-Esteves, H., Maldonado, H., Aguilar-León, D., Vilchis-Landeros, M., Mata-Espinosa, D., Mendoza, V. and López-Casillas, F., 2006. A combination of a transforming growth factor-β antagonist and an inhibitor of cyclooxygenase is an effective treatment for murine pulmonary tuberculosis. Clin Exper Immunol. 144, 264-272.

Hrabec, E., Strek, M., Zieba, M., Kwiatkowska, S. and Hrabec, Z., 2002. Circulating level of matrix metalloproteinase-9 is correlated with disease severity in tuberculosis patients. Int J Tuberc Lung Dis. 6, 713-719.

Hsu, C. and Patel, S., 1997. Uremic toxins and vitamin D metabolism. Kidney Int. Suppl 62, S65-S68.

Huff, S., 1963. Diminished tuberculin reaction after oral calciferol (vitamin D_2). Arch Dermatol. 88, 89-96.

Husain, N., Awasthi, S., Haris, M., Gupta, R. and Husain, M., 2008. Vascular endothelial growth factor as a marker of disease activity in neurotuberculosis. J Infect. 56, 114-119.

Jabado, N., Jankowski, A., Dougaparsad, S., Picard, V., Grinstein, S. and Gros, P. 2000. Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. J Exp Med. 192, 1237-1248.

Jack, C., Jackson, M. and Hind, C., 1994. Circulating markers of free radical activity in patient with pulmonary tuberculosis. Tuberc Lung Dis. 75m 132-127.

Jee, S., Golub, J., Jo, J., Park, I., Ohrr, H. and Samet, J., 2009. Smoking and risk of tuberculosis incidence, mortality, and recurrence in south Korean men and women. Am J Epidemiol. 170, 1478-1485.

Jehan, F. and DeLuca, H., 2000. The mouse vitamin D receptor is mainly expressed through and Sp1-driven promoter *in vivo*. Arch Biochem Biophys. 377, 273-283.

Jiang, J., Liu, B., Nasca, P., Zeng, X., Chen, J., Zou, X., Wu, Y., Han, W., Zhao, P. and Li, J., 2009. Smoking and risk of death due to pulmonary tuberculosis: a case-control comparison in 103 population centers in China. Int J Tuberc Lung Dis. 12, 1530-1535.

Jorde, R., Saleh, F., Figenschau, Y., Kamycheva, E., Haug, E. and Sundsfjord, J., 2005. Serum parathyroid hormone (PTH) levels in smokers and non-smokers. The fifth Tronsø study. Eur J Endocrinol. 152, 39-45.

Kalkunte, S., Brard, L., Granai, C. and Swamy, N., 2005. Inhibition of angiogenesis by vitamin D-binding protein: characterization of anti-endothelial activity of DBP-maf. Angiogenesis. 8, 349-360.

Kaminskaia, G., Blonskaia, G. and Gedymin, L., 1990. Prostaglandin levels in the lung tissue of guinea pigs with tuberculosis treated with anticubercular drugs and indomethacin. Probl Tuberk. (10), 6-10. [Article in Russian]

Kaminskaia, G., Blonskaia, G., Omarove, T. and Lovacheva, O., 1991. Level and ration of prostaglandins group E and $F_{2(alpha)}$ in bronchoalveolar washes in pulmonary tuberculosis patients. Vopr Med Khim. 37, 71-73. [Article in Russian]

Kang, S., Li, X., Duell, E. and Voorhees, J., 1997. The retinoid X receptor agonist 9-cis-retinoic acid and the 24-hydrpxy;ase inhibitor ketoconazole increase activity of 1,25-dihydroxyvitamin D3 in human skin *in vivo*. J Invest Dermatol. 108, 513-518.

Kang, D., Lin, Y., Moreno, J., Randall, T. and Khader, S., 2011. Profiling early lung immune responses in the mouse model of tuberculosis. PLos One. 6, e16161.

Khoo, A., Chai, L., Koenen, H., Oosting, M., Steinmeyer, A., Zuegel, U., Joosten, I., Netea, M. and Van der Ven, A., 2011. Vitamin D3 down-regulates proinflammatory cytokine response to Mycobacterium tuberculosis through pattern recognition receptors while inducing protective cathelicidin production. Cytokine. 55, 294-300.

Kim, H., Park, M., Song, E., Park, H., Kwon, S., Han, S. and Shim, Y., 2005. Association of HLA-DR and HLA-DQ genes with susceptibility to pulmonary tuberculosis in Koreans: preliminary evidence of associations with drug resistance, disease severity, and disease recurrence. Hum Immunol. 66, 1074-1081.

Kimlin, M., Olds, W. and Moore, M., 2007. Location and vitamin D synthesis: is the hypothesis validated by geographical data? J Photochem Photobiol B. 86, 234-239.

Kisker, O., Onizuka, S., Becker, C., Fannon, M., Flynn, E., D'Amato, R., Zetter, B., Folkman, J., Ray, R., Swamy, N. and Pirie-Shepherd, S., 2003. Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. Neoplasia. 5, 32-40.

Krall, E. and Dawson-Hughes, B., 1999. Smoking increases bone loss and decreases intestinal calcium absorption. J Bone Miner Res. 14, 215-220.

Kreindler, J., Steele, C., Nguyen, N., Chan, Y., Pilewski, J., Alcorn, J., Vyas, Y,, Aujla, S., Finelli, P., Blanchard, M., Zeigler, S., Logar, A., Hartigan, E., Kurs-Lasky, M., Rockette, H., Ray, A. and Kolls, J., 2010. Vitamin D3 attenuates Th2 responses to *Aspergillus fumigatus* mounted by CD4+ T cells from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. J Clin Invest. 120, 3242-3254.

Kuo, H., Wang, Y., Wang, C., He, C., Lin, S., Lin, H., Liu, C., Huang, K., Hsieh, L. and Huang, C., 2008. Matrix metalloproteinase-1 polymorphism in Taiwanese patients with endobronchial tuberculosis. Tuberculosis (Edinb). 88, 262-267.

Kwiatkowska, S., Piasecka, G., Zieba, M., Piotrowski, W. and Nowak, D., 1999. Increased serum concentrations of conjugated diens and malondialdehyhyde in patients with pulmonary tuberculosis. Respir Med. 93, 272-276.

Kwiatkowska, S., Szkudlarek, U., Nowak, D. and Zieba, M., 2007. Elevated exhalation of hydrogen peroxide and circulating IL-18 in patients with pulmonary tuberculosis. Respir Med. 101, 574-580.

Ma, X., Dou, S., Wright, J.A., Reich, R.A., Teeter, L.D., El Sahly, H.M., Awe, R.J., Musser, J.M. and Graviss, E.A., 2002. 5' dinucleotide repeat polymorphism of NRAMP1 and susceptibility to tuberculosis among Caucasian patients in Houston, Texas. Int J Tuberc Lung Dis. 6, 818-823.

Macmicking, J., North, R., LaCourse, R., Mudgett, J., Shah, S. and Nathan, C., 1997. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proc Natl Acad Sci USA. 94, 5243-5248.

Mahasirimongkol, S., Yanai, H., Nishida, N., Ridruechai, C., Matsushita, I., Ohashi, J., Summanapan, S., Yamada, N., Moolphate, S., Chuchotaworn, C., Chaiprasert, A., Manosuthi, W., Kantipong, P., Kanitwittaya, S., Sura, T., Khusmith, S., Tokunaga, K., Sawanpanyalert, P. and Keicho, N., 2009. Genome-wide SNP-based linkage analysis of tuberculosis in Thais. Genes Immun. 10, 77-83.

Mahmoudzadeh-Niknam, G. and Fadavi, P., 2003. Allelic distribution of human leukocyte antigen in Iranian patients with pulmonary tuberculosis. Hum Immunol. 64, 124-129.

Makinskii, A., Baikeev, R. and Zalialiev, R., 2002. Markers of alteration of tissue structures as indicators of pulmonary tuberculosis activity. Probl Tuberk. (7), 39-42. [Article in Russian].

Mantell, D., Owens, P., Bundred, N., Mawer, E. and Canfield, A., 2000. 1α,25-dihydroxyvitamin D3 inhibits angiogenesis *in vitro* and *in vivo*. Circ Res. 87, 214-220.

Marashian, S., Farnia, P., Seyf, S., Anoosheh, S. and Velayati, A., 2010. Evaluating the role of vitamin D receptor polymorphisms on susceptibility to tuberculosis among Iranian patients: a case-control study. Tuberk Toraks. 58, 147-153.

Martineau, A., Wilkinson, R., Wilkinson, K., Newton, S., Kampmann, B., Hall, B., Packe, G., Davidson, R., Eldridge, S., Maunsell, Z., Rainbow, S., Berry, J. and Griffiths, C., 2007. A single dose of vitamin D enhances immunity to Mycobateria. Am J Respir Crit Care Med. 176, 208-213.

Martineau, A., Leandro, A., Anderson, S., Newton, S., Wilkinson, K., Nicol, M., Pienaar, S., Skolimowska, K., Rocha, M., Rolla, V., Levin, M., Davidson, R., Bremner, S., Griffiths, C., Eley, B., Bonecini-Almeida, M. and Wilkinson, R., 2010. Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. Eur Respir J. 35, 1106-1112.

Martineau, A., Nhamoyebonde, S., Oni, T., Rangaka, M., Marais, S., Bangani, N., Tsekela, R., Bashe, L., de Azevedo, V., Caldwell, J., Venton, T., Timms, P., Wilkinson, K. and Wilkinson. R., 2011. Reciprocal seasonal vaviation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. Proc Natl Acad Sci USA. 108, 19013-19017.

Matrashkin, A., Mes'ko, E., Pospelov, L., Eremeev, V., Khaĭby, S. and Chistiakova, N., 2002. Examining the distribution of HLA antigens in patients with tuberculosis and in healthy individuals in the Erza district, Reblic of Tyva. Probl Tuberl. (12), 15-16. [Article in Russian]

Matsuyama, W., Hashiguchi, T., Matsumuro, K., Iwami, F., Hirotsu, Y., Kawabata, M., Arimura, K. and Osame, M., 2000. Increased serum level of vascular endothelial growth factor in pulmonary tuberculosis. Am J Respir Crit Care Med. 162, 1120-1122.

Matsuyama, W., Hashiguchi. T., Umehara, F., Matsuura, E., Kawabata, M., Arimura, K., Maruyama, I. and Osame, M., 2001. Expression of vascular endothelial growth factor in tuberculous meningitis. J Neurol Sci. 186, 75-79.

Matheu, V., Bäck, O., Mondoc, E. and Issazadeh-Navikas, S., 2003. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. J Allergy Clin Immunol. 112, 585-592.

Mehra, N., Rajalingam, R., Mitra, D., Taneja, V. and Giphart, M., 1995. Variants of HLA-DR2/DR51 group haplotypes and susceptibility to tuberculoid leprosy and pulmonary tuberculosis in Asian Indians. Int J Lepr Other Mycobact Dis. 63, 241-248.

Merza, M., Farnia, P., Anoosheh, S., Varahram, M., Kazampour, M., Pajand, O., Saeif, S., Mirsaeidi, M., Masjedi, M., Velayati, A. and Hoffner, S., 2009. The NRAMP1, VDR and TNF-α gene polymorphisms in Iranian tuberculosis patient: the study on hos susceptibility. Brazilian J Infect Dis. 13, 252-256.

Mohammed, K., Nasreen, N., Hardwick, J., Van Horn, R., Sanders, K. and Antony, V., 2003. Mycobacteria induces pleural mesothelial permeability by down-regulating beta-catenin expression. Lung. 181, 57-66.

Möller, M., Nebel, A., Kwiatkowski, R., van Helden, P., Hoal, E. and Schreiber, S., 2007. Hog susceptibility to tuberculosis: CARD15 polymorphisms in a South African population. Mol Cell Probes. 21, 148-151.

Morcos, M., Gabr, A., Samuel, S., Kamel, M., el Baz, M., el Beshry, M. and Michail, R., 1998. Vitamin D administration to tuberculous children and its value. Boll Chim Farm. 137, 157-164.

Moreno, J., Krishnan, A., Swami, S., Nonn, L., Peehl, D. and Feldman, D., 2005. Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. Cancer Res. 65, 7917-7925.

Motsinger-Reif, A., Antas, P., Oki, N., Levy, S., Holland, S. and Sterling, T., 2010. Polymorphisms in IL-1β, vitamin D receptor Fok1, and Toll-like receptor 2 are associated with extrapulmonary tuberculosis. BMC Med Genet. 11, 37.

Najmi, N., Kaur, G., Sharma, S. and Mehra, N., 2010. Human toll-like receptor 4 polymorphisms TLR4 Asp299Gly and Thr399lle influence susceptibility and severity of pulmonary tuberculosis in the Asian Indian population. Tissue Antigens. 76, 102-109.

Nakagawa, K., Sasaki, Y., Kato, S., Kubodera, N. and Okano, T., 2005. 22-Oxa-1α,25-dihydroxyvitamin D3 inhibits metastasis and angiogenesis in lung cancer. Carcinogenesis. 26, 1044-1054.

Need, A., Kemp, A., Giles, N., Morris, H., Horowitz, M. and Nordin, B., 2002. Relationship between intestinal calcium absorption, serum vitamin D metabolites and smoking in postmenopausal women. Osteoporos Int. 13, 83-88.

Newport, M., Allen, A., Awomoyi, A., Dunstan, S., McKinney, E., Marchant, A. and Sirugo, G., 2004. The toll-like receptor 4 Asp299Gly variant: no influence on LPS responsiveness or susceptibility to pulmonary tuberculosis in the Gambia. Tuberculosis (Edinb). 84, 347-352.

Nibali, L., Parkar, M., D'Aiuto, F., Suvan, J., Brett, P., Griffiths, G., Rosin, M., Schwahn, C. and Tonetti, M., 2008. Vitamin D receptor polymorphism (-1056 TaqI) interacts with smoking for the presence and progression of periodontitis. J Clin Periodontol. 35, 561-567.

Niño-Moreno, P., Portales-Pérez, D., Hernández-Castro, B., Portales-Cervantes, L., Flores-Meraz, V., Baranda, L., Gómez-Gómez, A., Acuña-Alonzo, V., Granados, J. and González-Amaro, R., 2007. P2X7 and NRAMP1/SLC11 A1 gene polymorphisms in Mexican mestizo patients with pulmonary tuberculosis. Clin Exp Immunol. 148, 469-477.

Nisar, M., Williams, C., Ashby, D. and Davies, G., 1993. Tuberculin testing in residential homes for the elderly. Thorax. 48, 1257-1260.

Nnoaham, K. and Clarke, A., 2008. Low serum vitamin D levels and tuberculosis: a systemic review and meta-analysis. Int J Epidemiol. 37, 113-119.

North, R., 1988. Mice incapable of making IL-4 or IL-10 display normal resistance to infection with *Mycobacterium tuberculosis*. Clin Exp Immunol. 113, 55-58.

Nugraha, J. and Anggraini, R., 2011. NRAMP1 polymorphism and susceptibility to lung tuberculosis in Surabaya, Indonesia. Southeast Asian J Trop Med Public Health. 42, 338-341.

Nursyam, E., Amin, Z. and Rumende, C., 2006. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones. 38, 3-5.

Lacraz, S., Dayer, J., Nicod, L. and Welgus, H., 1994. 1,25-dihydroxyvitamin D3 dissociates production of interstitial collagenase and 92-kDa gelatinase in human mononuclear phagocytes. J Biol Chem. 269, 6485-6490.

Ladel, C., Daugelat, S. and Kaufmann, S., 1995. Immune response to Mycobacterium bovis bacilli Calmette Guérin infection in major histocompatibility complex class I- and II-deficient knock-out mice: contribution of CD4 and CD8 T cells to acquired resistance. Eur J Immunol. 25, 377-284.

Lalor, M., Floyd, S., Gorak-Stolinska, P., Weir, R., Blitz, R., Branson, K., Fine, P. and Dockrell, H., 2011. BCG vaccination: a role for vitamin D? PLos ONE. 6, 216709.

Larcombe, L., Orr, P., Lodge, A., Brown, J., Dembinski, I., Milligan, L., Larcombe, E., Martin, B. and Nickerson, P., 2008. Functional gene polymorphisms in Canadian Aboriginal populations with high rates of tuberculosis. J Infect Dis. 198, 1175-1179.

Lauridsen, A., Vestergaard, P., Hermann, A., Brot, C., Heickendorff, L., Mosekilde, L. and Nexo, E., 2005. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxyvitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int. 77, 15-22.

Lehmann, B., Knuschke, P. and Meurer, M., 2000. UVB-induced conversion of 7-dehydrocholesterol to 1α,25-dihydroxyvitamin D3 (Calcitriol) in the human keratinocyte line HaCaT. Photochem Photobiol. 72, 803-809.

Leung, C., Lam, T., Ho, K., Yew, W., Tam, C., Chan, W., Law, W., Chan, C., Chang, K. and Au, K., 2010. Passive smoking and tuberculosis. Arch Intern Med. 170, 287-292.

Levy, R. and Malech, H., 1991. Effect of 1α-dihydroxyvitamin D3, lipoxysaccharide, or lipoteichoic acid on the expression of NADPH oxidase components in cultured human monocytes. J Immunol. 147, 3066-3071.

Liaw, Y., Tsai-Wu, J., Wu, C., Hung, C., Lee, C., Yang, P., Luh, K. and Kuo, S., 2002. Variations in the NRAMP1 gene and susceptibility of tuberculosis in Taiwanese. Int J Tuberc Lung Dis. 6, 454-460.

Li, X., Yang, Y., Zhou, F., Zhang, Y., Lu, H., Jin, Q. and Gao, L., 2011. SLC11A1 (NRAMP1) polymorphisms and tuberculosis susceptibility: updated systematic review and meta-analysis. PLos ONE. 6, e15831.

Liu, Z., Luo, Y., Zhou, L., Xu, W., Feng, D., Tan, Y., He, X., Song, C., Fu, Y. and Liu, Z., 2004a. A study on the correlation between HLA-DR genes and susceptibility to pulmonary tuberculosis in a population of Han nationality from southern China. Zhonghua Jie He He Hu Xi Za Zhi. 27, 390-393. [Article in Chinese]

Liu, W., Cao, W., Zhang, C., Tian, L., Wu, X., Habbema, J., Zhao, Q., Zhang, P., Xin, Z., Li, C. and Yang, H., 2004b. VDR and NRAMP1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the Chinese Han population: a case-control study. 8, 428-434.

Liu, P., Stenger, S., Li, H., Wenzel, L., Tan, B., Krutzik, S., Ochoa, M., Schauber, J., Wu, K., Meinken, C., Kamen, D., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R., Eisenberg, D., Hewison, M., Hollis, B., Adams, J., Bloom, B. and Modlin, R., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 311, 1770-1773.

Liu, P., Stenger, S., Tang, D. and Modlin, R., 2007. Vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. J Immunol. 179, 2060-2063.

Liu, P., Schenk, M., Walker, V., Dempsey, P., Kanchanapoomi, M., Wheelwright, M., Vazirnia, A., Zhang, X., Steinmeyer, A., Zügel, U., Hollis, B., Cheng, G. and Modlin, R., 2009. Convergence of IL-1beta and VDR activation pathway in human TLR2/1-induced antimicrobial responses. PLoS One. 4, e5810.

Lombard, Z., Dalton, D., Venter, P., Williams, R. and Bornman, L., 2006. Association of HLA-DR, -DQ and vitamin D receptor alleles and haplotypes with tuberculosis in the Venda of South Africa. Hum Immunol. 67, 643-654.

Lorentzon, M., Mellström, D., Haug, E. and Ohlsson, C., 2007. Smoking is associated with lower bone mineral density and reduced cortical thickness in young men. J Clin Endocrinol Metab. 92, 497-503.

Lowrie, D. and Andrew, P., 1988. Macrophage antimycobacterial mechanisms. Br Med Bull. 44, 624-634.

Lương, K. and Nguyễn, L., 2011. Impact of vitamin D in the treatment of tuberculosis. Am J Med Sci. 341, 493-498.

O'Leary, T., Jones, G., Yip, A., Lohnes, D., Cohanim, M. and Yendt, E., 1986. The effect of chloroquine on serum 1,25-dihydroxyvitamin D3 and calcium metabolism in sarcoidosis. N Engl J Med. 315, 727-730.

Oliveri, M., Ladizesky, M., Mautalen, C., Alonso, A. and Martinez, L., 1993. Seasonal variations of 25-hydroxyvitamin D and parathyroid hormone in Ushuala (Argentina), the southernmost city of the world. Bone Miner. 20, 99-108.

Pattison, C., 1929. Vitamin D and the calcification of bone in tuberculosis. Br Med J. 419-420.

Peehl, D., Seto, E., Hsu, J. and Feldman, D., 2002. Preclinical activity of ketoconazole in combination with calcitriol or the vitamin D analogues EB 1089 in prostate cancer cells. J. Urol. 168, 1583-1588.

Prabha, C., Rajashree, P. and Das, S., 2008. TLR2 and TLR4 expression on the immune cells of tuberculous pleural fluid. Immunol Lett. 117, 26-34.

Pulido, I., Leal, M., Genebat, M., Pacheco, Y., Sáez, M. and Soriano-Sarabia, N., 2010. The TLR4 ASP299GLY polymorphism is a risk factor for active tuberculosis in Caucasian HIV-infected patients. Curr HIV Res. 8, 253-258.

Racil, H., Amar, J., Cheikrouhou, S., Hassine, E., Zarrouk, M., Chaouch, N. and Chabbou, A., 2010. Pulmonary tuberculosis in smokers. Presse Med. 39, e25-28. [Article in French]

Ragno, S., Romano, M., Howell, S., Pappin, D., Jenner, P. and Colston, M., 2011. Changes in gene expression in macrophages infected with Mycobacterium tuberculosis: a combined transcriptomic and proteomic approach. Immunology. 104:99-108.

Randhawa, A., Shey, M,, Keyser, A., Peixoto, B., Wells, R., de Kock, M., Lerumo, L., Hughes, J., Hussey, G., Hawkridge, A., Kaplan, G., Hanekom, W., Hawn, T. and South African Tuberculosis Vaccine Initiative Team, 2011. Association of human TLR1 and TLR6 deficiency with altered immune responses to BCG vaccination in South African infants. PLoS Pathog. 7:e1002174.

Range, N., Changalucha, J., Krarup, H., Magnussen, P., Andersen, A. and Friis, H., 2006. The effect of multivitamin/mineral supplementation on mortality during treatment of pulmonary tuberculosis: a randomized two-by-two factorial trial in Mwanza, Tanzania. Br J Nutr. 95, 762-770.

Ravikumar, M., Dheenadhayalan, V., Rajaram, K., Lakshmi, S., Kumaran, P., Paramasivan, C., Balakrishnan, K. and Pitchappan, R., 1999. Associations of HLA-DRB1, DQB1 and DPB1 alleles with pulmonary tuberculosis in south India. Tuberc Lung Dis. 79, 309-317.

Rhodes, S., Terry, L., Hope, J., Hewinson, R. and Vordermeier, H., 2003. 1,25-dihydroxyvitamin D3 and development of tuberculosis in cattle. Clin Diagn Lab Immunol. 10, 1129-1135.

Richer, E., Campion, C., Daddas, B., White, J. and Cellier, M., 2008. Transcription factors Sp1 and C/EBP regulate NRAMP1 gene expression. FEBS J. 275, 5074-5089.

Rigby, W., Waugh, M. and Graziano, R., 1990. Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25-dihydroxyvitamin D3. Blood. 76, 189-197.

Rivas-Santiago, B., Hernandez-Pando, R., Carranza, C., Juarez, E., Contreras, J., Aguilar-Leon, D,, Torres. M, and Sada. E., 2008. Expression of cathelicidin LL-37 during *Mycobacterium tuberculosis* infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. Infect Immunity. 76, 935-941.

Rockett, K., Brookes, R., Udalova, I., Vidal, V., Hill, A. and Kwiatkowski, D., 1998. 1,25-hydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of *Mycobacterium tuberculosis* in a human macrophage-like cell line. Infect Immun. 66, 5314-5321.

Roig, E., Richer, E., Canonne-Hergaux, F., Gros, P. and Cellier, M., 2002. Regulation of NRAMP1 gene expression by 1α,25-dihydroxyvitamin D3 in HL-60 phagocytes. J Leukoc Biol. 71, 890-904.

Rook, G., 1988a. Role of activated macrophages in the immunopathology of tuberculosis. Br Med Bull. 44, 611-623.

Rook, G., 1988b. The role of vitamin D in tuberculosis. Am Rev Respir Dis. 138, 768-770.

Roth, D., Soto, G., Arenas, F., Bautista, C., Ortiz, J., Rodriguez, R., Cabrera, L. and Gilman, R., 2004. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis. 190, 920-927.

Ryu, S., Park, Y., Bai, G., Kim, S., Park, S. and Kang, S., 2000. 3'UTR polymorphisms in the NRAMP1 gene are associated with susceptibility to tuberculosis in Koreans. Int J Tuberc Lung Dis. 4, 577-580.

Sadeghi, K., Wessner, B., Laggner, U., Ploder, M., Tamandl, D., Friedl, J., Zügel, U., Steinmeyer, A., Pollak, A., Roth, E., Boltz-Nitulescu, G. and Spittler, A., 2006. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur J Immunol. 36, 361-370.

Safarian, M. and Karapetian, E., 1990. Dynamics of activity of antioxidant enzymes in the blood of patients with pulmonary tuberculosis. Probl Tuberk. (8), 60-61. [Article in Russian]

Saggese, G., Bertelloni, S., Baroncelli, G. and Di Nero, G., 1993. Ketoconazole decreases the serum ionized calcium and 1,25-dihydroxyvitamin D levels in tuberculous-associated hypercalcemia. Am J Dis Child. 147, 270-273.

Sakurai, T., Tamura, K. and Kogo, H., 2004. Vascular endothelial growth factor increases messenger RNAs encoding cyclooxygenase-II and membrane associated E synthase in rat luteal cells. J Endocrinol. 183, 527-533.

Sánchez, D., Rojas, M., Hernández, I., Radzioch, D., García, I. and Barrera, L., 2010. Role of TLR2- and TLR4-mediated signaling in *Mycobactrium tuberculosis*-induced macrophage death. Cell Immunol. 260, 128-136.

Sardar, S., Chakraborty, A. and Chatterjee, M., 1996. Comparative effectiveness of vitamin D3 and dietary vitamin E on peroxidation of lipids and enzymes of the hepatic antioxidant system in Sprague-Dawley rats. Int J Vitam Nutr Res. 66, 39-45.

Schaaf, H., Nel, E., Beyers, N., Gie, R., Scott, F. and Donald, P., 1996. A decade of experience with *Mycobacterium tuberculosis* culture from children: a seasonal influence on incidence of childhood tuberculosis. Tuberc Lung Dis. 77, 43-46.

Scherberich, J., Kellermeyer, M., Ried, C. and Hartinger, A., 2005. 1-alpha-calcidol modulates major human monocyte antigens and toll-like receptors TRL2 and TRL4 *in vitro*. Eur J Med Res. 10, 179-182.

Seah, G., Scott, G. and Rook, G., 2000. Type 2 cytokine gene activation and its relationship to extent of disease in patients with tuberculosis. J Infect Dis. 181, 385-389.

Seiscento, M., Vargas, F., Acencio, M., Teixeira, L., Capelozzi, R., Sales, R. and Antonangelo, L., 2010. Pleural fluid cytokines correlate with tissue inflammatory expression in tuberculosis. Int J Tuberc Lung Dis. 14, 1153-1158.

Selvaraj, P., Kurian, S., Umam, H., Reetha, A. and Narayanan, P., 2000a. Influence of non-MHC genes on lymphocyte response to Mycobacterium tuberculosis antigens & tuberculin reactive status in pulmonary tuberculosis. Indian J Med Res. 112, 86-92.

Selvaraj, P., Narayanan, P. and Reetha, A., 2000b. Association of vitamin D receptor genotypes with the susceptibility to pulmonary tuberculosis in female patients & resistance in female contacts. Indian J Med Res. 111, 172-179.

Selvaraj, P., Kurian, S., Chandra, G., Reetha, A., Charles, N. and Narayanan, P., 2003. Association of vitamin D receptor gene variants of BsmI, ApaI and FokI polymorphisms with susceptibility or resistance to pulmonary tuberculosis. Curr Sci. 84, 1564-1568.

Selvaraj, P., Alagarasu, K., Harishankar, M., Vidyarani, M. and Narayanan, P., 2008. Regulatory region polymorphisms of vitamin D receptor gene in pulmonary tuberculosis patients and normal healthy subjects on south India. Int J Immunogenet. 35, 251-254.

Selvaraj, P., Prabhu Anand, S., Harishankar, M. and Alagarasu, K., 2009. Plasma 1,15 dihydroxyvitamin D3 level and expression of vitamin D receptor and cathelicidin in pulmonary tuberculosis. J Clin Immunol. 29, 470-478.

Selvaraj, P., Harishankar, M., Singh, B., Jawahar, M. and Banurekha, V., 2010. Toll-like receptor and TIRAP gene polymorphisms in pulmonary tuberculosis patients in South India. Tuberculosis (Edinb). 90, 306-310.

Sharma, P., Singh, S., Jena, M., Mishra, G., Prakash, R., Das, P., Bamezai, R. and Tiwari, P., 2011. Coding and non-coding polymorphisms in VDR gene and susceptibility to pulmonary in tribes, castes, and Muslims of Central India. Infect Genet Evolut. 11, 1456-1461.

Sheen, P., O'Kane. C., Chaudhary, K., Tovar, M., Santillan, C., Sosa, J., Caviedes, L., Gilman, R., Stamp, G. and Friedland, J., 2009. High MMP-9 activity characterizes pleural tuberculosis correlating with granuloma. 33, 134-141.

Shey, M., Randhawa, A., Bowmaker, M., Smith, E., Scriba, T., de Kock, M., Mahomed, H., Hussey, G., Hawn, T. and Hanekom, W., 2010. Single nucleotide polymorphisms in toll-like receptor 6 are associated with altered lipopeptide- and mycobacteria-induced interleukin-6 secretion. Genes Immun. 11, 561-572.

Shi, G., Hu, X., Yang, L., Rong, C., Guo, Y. and Song, C., 2011. Association of HLA-DRB alleles and pulmonary tuberculosis in North Chinese patients. Genet Mol Res. 10, 1331-1336.

Shilova, M. and Glumnaia, T., 2004. Influence of seasonal and environmental factors on the incidence of tuberculosis. Probl Tuberk Bolezn Legk. 2, 17-22. [Article in Russia]

Shim, T., Turner, O. and Orme, I., 2003. Toll-like receptor 4 plays no role in susceptibility of mice to Mycobacterium tuberculosis infection. Tuberculosis (Edinb). 83, 367-371.

Shiryaev, S., Cieplak, P., Aleshin, A., Sun, Q., Zhu, W., Motamedchaboki, K., Sloutsky, A. and Strongin, A., 2011. Matrix metalloproteinase proteolysis of the mycobacterial HSP65 protein as a potential source of immunogenic peptides in human tuberculosis. 278, 3277-3286.

Sita-Lunsden, A., Lapthorn, G., Swaminathan, R. and Milburn, H., 2009. Reactivation of tuberculosis and vitamin D deficiency: the contribution of diet and exposure to sunlight. Thorax. 62, 1003-1007.

Slama, K., Chiang, C., Enarson, D., Hassmiller, K., Fanning, A., Gupta, P. and Ray, C., 2007. Tobacco and tuberculosis: a qualitative systematic review and meta-analysis. Int J Tuberc Lung Dis. 11, 1049-1061.

Somjen, D., Katzburg, S., Grafi-Cohen, M., Knoll, E., Sharon, O. and Posner, G., 2011. Vitamin D metabolites and analogs induce lipoxygenase mRNA expression and as well as reactive oxygen species (ROS) production in human bone cell line. J Steroid Biochem Mol Biol. 123, 85-89.

Stagas, M., Papaetis, G., Orphanidou, D., Kostopoulos, C., Syriou, S., Reczko, M. and Drakoulis, N., 2011. Polymorphisms of the NRAMP1 gene: distribution and susceptibility to the development of pulmonary tuberculosis in the Greek population. Med Sci Monit. 17, PH1-6.

Strachan, D., Powell, K., Thaker, A., Millard, F. and Maxwell, J., 1995. Vegeterian diet is a risk factor for tuberculosis in immigrant south London Asians. Thorax. 50, 175-180.

Stockton, J., Howson, J., Awomoyi, A., McAdam, K., Blackwell, J. and Newport, M., 2004. Polymorphism in NOD2, Crohn's disease, and susceptibility to pulmonary tuberculosis. FEMS Immunol Med Microbiol. 41, 157-160.

Stryd, R., Gilbertson, T. and Bruden, M., 1979. A seasonal variation study of 25-hydroxyvitamin D3 serum levels in normal human. J Clin Endocrinol Metab. 48, 771-775.

Sundar, I., Hwang, J., Wu, S., Sun, J. and Rahman, I., 2011. Deletion of vitamin D receptor leads to premature emphysema/COPD by increased matrix metalloproteinase and lymphoid aggregates formation. Biochem Biophys Res Commun. 406, 127-133.

Supervía, A., Nogués, X., Enjuanes, A., Vila, J., Mellibovsky, L., Serrano, S., Aubía, J. and Díez-Pérez, A., 2006. Effect of smoking and smoking cessation on bone mass, bone remodeling, vitamin D, PTH and sex hormones. J Musculoskelet Neuronal Interact. 6, 234-241.

Tachfouti, N., Nejjari, C., Benjelloun, M., Berraho, M., Elfakir, S., El Rhazi, K. and Slama, K., 2011. Association between smoking status, other factors and tuberculosis treatment failure in Morocco. Int J Tuberc Lung Dis. 15, 838-843.

Tamaki, K., Saitoh, A. and Kubota, Y., 1990-1991. 1,25-hydroxyvitamin D3 decreases the interferon-gamma (IFN-gamma) induced HLA-DR expression but not intercellular adhesion molecule 1 (ICAM-1) on human keratinocytes. Reg Immunol. 3, 223-227.

Tan, Q., Xie, W., Min, R., Dai, G., Xu, C., Pan, H., Miao, C., Yang, Z., Xu, W. and Wang, H., 2012. Characterization of Th1- and Th2-type immune response in human multidrug-resistant tuberculosis. Eur J Clin Microbiol Infect Dis. 31, 1233-1242.

Talat, N., Perry, S., Parsonnet, J., Dawood, G. and Hussain, R., 2010. Vitamin D deficiency and tuberculosis progression. Emerg Infect Dis. 16, 853-855.

Teixeira-Coelho, M., Cruz, A., Carmona, J., Sousa, C., Ramos-Pereira, D., Saraiva, A., Veldhoen, M,, Pedrosa, J., Castro, A. and Saraiva, M., 2011. TLR2 deficiency by compromising p19 (IL-23) expression limits Th17 cell responses to *Mycobacterium tuberculosis*. Int Immunol. 23, 89-96.

Terán-Escandón, D., Terán-Ortiz, L., Camarena-Olvera, A., González-Avila, G., Vaca-Marín, M., Granados, J. and Selman, M., 1999. Human leukocyte antigen-associated susceptibility to pulmonary tuberculosis: molecular analysis of class II alleles by DNA amplification and oligonucleotide hybridization in Mexican patients. Chest. 115, 428-433.

Thuong, N., Hawn, T., Thwaites, G., Chau, T., Lan, N., Quy, H., Hieu, N., Aderem, A., Hien, T., Farrar, J. and Dunstan, S.,. 2007. A polymorphism in human TLR2 is associated with increased susceptibility to tuberculous meningitis. Genes Immun. 8, 422-428.

Timms, P., Mannan, N., Hitman, G., Noonan, K/, Mills. P., Syndercombe-Court, D., Aganna, E., Price, C. and Boucher, B., 2002. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? Q J Med. 95, 787-796.

Tokuda, N., Mizuki, N., Kasahara, M. and Levy, R., 1992. 1,25-hydroxyvitamin D3 down-regulation of HLA-DR on human peripheral blood monocytes. Immunology. 75, 349-354.

Tokuda, N. and Levy, R., 1996. 1,25-hydroxyvitamin D3 stimulates phagocytosis but suppresses HLA-DR and CD13 antigen expression in human mononuclear phagocytes. Proc Soc Exp Biol Med. 211, 244-250.

Tone, T., Eto, H., Katsuoka, K., Nishioka, K. and Nishiyama, S., 1991. Suppression of gamma-interferon induced HLA-DR antigen expression on normal and transformed keratinocytes by 1,25 $(OH)_2$ vitamin D3. Nippon Hifuka Gakkai Zasshi. 101, 519-525 [Article in Japanese].

Tone, T., Eto, H., Katou, T., Otani, F. and Nishiyama, S., 1993. 1 alpha,25-dihydroxy vitamin D3 modulation of HLA-DR mRNA induced by gamma-interferon in cultured epithelial tumor cell lines. J Dermatol. 20, 581-584.

Topilski, I., Flaishon, L., Naveh, Y., Harmelin, A., Levo, Y. and Shachar, I., 2004. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells *in vivo* are due in part to the control of integrin-mediated T lymphocyte homing. Eur J Immunol. 34, 1068-1076.

Tostmann, A., Wielders, J., Kibiki, G., Verhoef, H., Boeree, M. and van der Ven, A., 2010. Serum 25-hydroxy-vitamin D3 concentrations increase during tuberculosis treatment in Tanzania. Int J Tuberc Lung Dis. 14, 1147-1152.

Trifunović, V., Gvozdenović, B., Jovanovic, D., Vucinić, V., Djurić, D. and Zunić, M., 2009. Association between tobacco smokine and pulmonary tuberculosis. Vojnosanit Pregl. 66, 797-801. [Article in Serbian]

Underhill, D., Ozinsky, A., Smith, K. and Aderem, A., 1999. Toll-like receptor-2 mediated mycobacteria-induced proinflammatory signaling in macrophages. Proc Natl Acad Sci USA. 98, 14459-14463.

Vantieghem, K., Kissmeyer, A., Haes, P., Bouillon, R. and Segaert, S., 2006. UVB-induced production of 1,25α-dihydroxyvitamin D3 and vitamin D activity in human keratinocytes pretreated with a sterol Δ^7-reductase inhibitor. J Cell Biochem. 98, 81-92.

Vejbaesya, S., Chierakul, N., Luangtrakool, K., Srinak, D. and Stephens, H., 2002. Associations of HLA class II alleles with pulmonary tuberculosis in Thais. Eur J Immunogenet. 29, 431-434.

Vejbaesya, S., Chierakul, N., Luangtrakool, P. and Sermduangprateep, C., 2007. NRAMP1 and TNF-alpha polymorphisms and susceptibility to tuberculosis in Thais. Respirology. 12, 202-206.

Velez, D., Wejse, C., Stryjewski, M., Abbate, E., Hulme, W., Myers, J., Estevan, R., Patillo, S., Olesen, R., Tacconelli, A., Sirugo, G., Gilbert, J., Hamilton, C. and Scott, W., 2010. Variants in toll-like receptors 2 and 9 influence susceptibility to pulmonary tuberculosis in Caucasians, African-Americans, and West Africans. Hum Genet. 127, 65-73.

Vidal, S., Tremblay, M., Govoni, G., Gauthier, S., Sebastiani, G., Malo, D., Skamene, E., Olivier, M., Jothy, S. and Gros, P., 1995. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1. J Exp Med. 182, 655-666.

Wade, M. and Zhang, Y., 2006. Effects of weak acids, UV and proton motive force inhibitors on pyrazinamide activity against *Mycobacterium tuberculosis in vitro*. J Antimicrob Chemother. 58, 936-941.

Wang, J. and Shen, H, 2009. Review of cigarette smoking and tuberculosis in China: intervention is needed for smoking cessation among tuberculosis patients. BMC Public Health. 9, 292.

Wang, T., Zhang, F., Richards, J., Kestenbaum, B., van Meurs. J., Berry, D., Kiel, D., Streeten, E., Ohlsson, C., Koller, D., Peltonen, L., Cooper, J., O'Reilly, P., Houston, D., Glazer, N., Vandenput, L., Peacock, M., Shi, J., Rivadeneira, F., McCarthy, M., Anneli, P., de Boer, I., Mangino, M., Kato, B., Smyth, D., Booth, S., Jacques, P., Burke, G., Goodarzi, M., Cheung, C., Wolf, M., Rice, K., Goltzman, D., Hidiroglou, N., Ladouceur, M., Wareham, N., Hocking, L., Hart, D., Arden, N., Cooper, C., Malik, S., Fraser, W., Hartikainen, A., Zhai, G., Macdonald, H., Forouhi, N., Loos, R., Reid, D., Hakim, A., Dennison, E., Liu, Y., Power, C., Stevens, H., Jaana, L., Vasan, R.,

Soranzo, N., Bojunga, J., Psaty, B., Lorentzon, M., Foroud, T., Harris, T., Hofman, A., Jansson, J., Cauley, J., Uitterlinden, A., Gibson, Q., Järvelin, M., Karasik, D., Siscovick, D., Econs, M., Kritchevsky, S., Florez, J., Todd, J., Dupuis, J., Hyppönen, E. and Spector, T., 2010a. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 376, 180-188.

Wang, T., Dabbas, B., Laperriere, D., Bitton, A., Soualhine, H., Tavera-Mendoza, L., Dionne, S., Servant, M., Bitton, A., Seidman, E., Mader, S., Behr, M. and White, J., 2010b. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-Defensin β2 innate immune pathway defective in Crohn disease. J Biol Chem. 285, 2227-2231.

Wang, C., Lin, H., Lin, S., Huang, C., Liu, C., Huang, K., Hsieh, L., Chung, K. and Kuo, H., 2010c. MMP-1(-1607G) polymorphism as a risk factor for fibrosis after pulmonary tuberculosis in Taiwan. Int J Tuberc Lung Dis. 14, 627-634.

Warren, P., 2006. The evolution of the sanatorium: the first half-century, 1854-1904. Can Bull Med Hist. 23:457-476.

Webb, A., Kline, L. and Holick, M., 1988. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 67, 373-378.

Wejse, C., Gomes, V., Rabna, P., Gustafson, P., Aaby, P., Lisse, I., Andersen, P., Glerup, H. and Sodemann, M., 2009. Vitamin D supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med. 179, 843-850.

Wen, C., Chan, T., Chan, H, Tsai, M, Cheng, T. and Tsai, S., 2010. The reduction of tuberculosis risks by smoking cessation. BMC Infect Dis. 10:156.

Wilbur, A., Kubatko, L., Hurtado, A., Hill, K. and Stone, A., 2007. Vitamin D receptor gene polymorphisms and susceptibility *M. tuberculosis* in native Paraguayans. Tuberculosis (Edinb). 87, 329-337.

Wilkinson, R., Llewelyn, M., Toossi, Z., Patel, P., Pasvol, G., Lalvani, A., Wright, D., Latif, M. and Davidson, RN., 2000. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet. 355, 618-621.

Xu, H., Soruri, A., Gieseler, R. and Peters, J., 1993. 1,25-Dihydroxyvitamin D3 exerts opposing effects to IL-4 on MHC class-II antigen expression, accessory activity, and phagocytosis of human monocytes. Scand J Immunol. 38, 535-540.

Xue, Y., Jin, L., Li, A., Wang, H., Li, M., Zhang, Y., Wang, Y. and Li, J., 2010a. Microsatellite polymorphisms in intron 2 of the toll-like receptor 2 gene and their association with susceptibility to pulmonary tuberculosis in Han Chinese. Clin Chem Lab Med. 48, 785-789.

Xue, Y., Zhao, Z., Wang, H., Jin, L., Liu, C., Wang, Y. and Li, J., 2010b. Toll-like receptors 2 and 4 gene polymorphisms in a southeastern Chinese population with tuberculosis. Int J Immunogenet. 37, 135-138.

Yamshchikov, A., Oladele, A., Leonard, M. Jr, Blumberg, H., Ziegler, T. and Tangpricha, V., 2009. Vitamin D as adjunctive therapy in refractory pulmonary tuberculosis: a case report. South Med J. 102, 649-652.

Yesudian, P., Berry, J., Wiles, S., Hoyle, S., Young, D., Haylett, A., Rhodes, L. and Davies, P., 2008. The effect of ultraviolet B-induced vitamin D levels on host resistance to *Mycobacterium tuberculosis*: a pilot study in immigrant Asian adults living in the United Kingdom. Photodermatol Photoimmunol Photomed. 24, 97-98.

Yim, J., Lee, H., Lee, H., Kim, Y., Han, S., Shim, Y. and Holland, S., 2006. The association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and tuberculosis among Koreans. Genes Immun. 7:150-155.

Yim, S., Dhawan, P., Ragunath, C., Christakos, S. and Diamond, G., 2007. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D3. J Cys Fibros. 6, 403-410.

Yuk, J., Shin, D., Lee, H., Yang, C., Jin, H., Kim, K., Lee, Z., Lee, S., Kim, J. and Jo, E., 2009. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microb. 6, 231-243.

Yuliwulandari, R., Sachrowardi, Q., Nakajima, H., Kashiwase, K., Hirayasu, K., Mabuchi, A., Sofro, A. and Tokunaga, K., 2010. Association of HLA-A, -B, and –DRB1 with pulmonary tuberculosis in western Javanese Indonesia. Hum Immunol. 71, 697-701.

Zhang, W., Shao, L., Weng, X., Hu, Z., Jin, A., Chen, S., Pang, M. and Chen, Z., 2005. Variants of the natural resistance-associated macrophage protein 1 gene (NRAMP1) are associated with severe forms of pulmonary tuberculosis. Clin Infect Dis. 40:1232-1236.

Zhang, H., Deng, A., Guo, C., Wang, Y., Chen, L., Wang, Y., Wu, J. and Liu, J., 2010. Association between FokI polymorphism in vitamin D receptor gene and susceptibility to spinal tuberculosis in Chinese Han population. Arch Med Res. 41, 46-49.

Zhao, Z., Zhang, T., Gao, Y. and Feng, F., 2009. Meta-analysis of relationship of vitamin D receptor gene polymorphism and tuberculosis susceptibility. Zhonghua Jie He He Hu Xi Za Zhi. 32, 748-751 [Article in Chinese].

Key facts

- Periodontal disease is common and includes gingivitis (inflammation of the soft tissues surrounding teeth) and periodontitis (inflammation and destruction of soft tissue and bone surrounding teeth).
- According to the 2000 US Surgeon General report, dental caries (infection and destruction of tooth structure) is the single most common chronic childhood disease.
- Inadequate levels of vitamin D may increase the risk for developing dental caries and periodontal disease.
- Diseases of the oral cavity have multifactorial causes.
- Vitamin D is necessary for the mineralization of tissues in the oral cavity including bone and teeth.

Summary points

- Gingivitis is an inflammatory condition confined to the soft tissues surrounding teeth that does not result in destruction of bone or the attachment apparatus.
- Vitamin D receptors are found in cells involved with calcium and bone metabolism, and immune regulation, and these receptors are present in cells of the oral cavity.
- Periodontitis is an inflammatory infectious disease of the supporting tissues of the teeth and results in destruction of bone and connective tissue attachment around the tooth.
- There is a growing body of evidence to suggest that vitamin D contributes directly to bone strength and plays a role in host immunity, both of which can alter the pathogenesis of gingivitis and periodontitis.
- Vitamin D levels can affect the calcification of tooth enamel of permanent teeth, the rate of tooth eruption, and the position of teeth.
- Caries incidence may be related to indirect vitamin D levels such as diet and sunlight exposure.
- High levels of vitamin D may have protective effects on bone and teeth in the oral cavity.
- Vitamin D toxicity may adversely affect tooth morphology and oral bone development.

19. Vitamin D and dental health

E. Jordan, S. Davis, P. Rakes, L.K. McCauley and J. Bashutski
Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, 1011 North University Avenue, Ann Arbor, MI 48109-1078, USA; jillbash@umich.edu

Abstract

Common oral diseases include gingivitis (inflammation of soft 'gum' tissue), periodontitis (infection of hard and soft tissues around teeth) and caries (infection of teeth). These conditions are influenced by the body's immune function as well as its ability to promote mineralization of hard tissues in the area. As a mediator of mineralization and immunity, vitamin D levels may influence these conditions. Vitamin D has anti-inflammatory and anti-bacterial roles in the oral cavity. Its anti-inflammatory effects are a result of many different interactions including activation of both acquired and innate immunity. The presence of vitamin D also provides indirect antimicrobial effects through increasing cathelicidin levels, which destroy both gram negative and gram positive bacteria. As a result, vitamin D deficiency may compromise immunity and allow bacterial growth within the oral cavity, ultimately increasing local inflammation and the development of periodontal disease. Vitamin D is also necessary for mineralization of dental tissues including alveolar bone, enamel, and dentin. Inadequate mineralization of these tissues as a result of vitamin D deficiency can contribute to decreased integrity of these structures, placing them at higher risk for disease. In the presence of inflammation and pathogenic bacteria, which is also exacerbated by vitamin D deficiency, oral conditions such as periodontitis and caries may be at higher risk of development. While most evidence shows an association between vitamin D deficiency and conditions of the oral cavity, it is important to remember that oral diseases are often multifactorial. Evidence linking vitamin D deficiency with increased risk for oral disease requires more investigation to show causality and determine the magnitude of the contribution. Vitamin D is involved in many vital aspects of mineralization, immunity, and tissue integrity that suggest a key role in preventing oral disease.

Keywords: caries, gingivitis, periodontitis, vitamin D toxicity

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_19, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
AP	Agresive periodontitis
BOP	Bleeding on probing
CP	Chronic periodontitis
DEFT	Decayed, extracted and filled teeth
NHANES	National Health and Nutrition Examination Survey
OPG	Osteoprotegerin
RCE	Reported-caries experience
VDR	Vitamin D receptor

19.1. Introduction

Establishing and maintaining health of the oral cavity requires harmony of various factors related to one's habits, genetics and systemic health. For the majority of people, oral and dental health can be maintained through brushing and flossing in addition to regular prophylactic cleanings and exams with an oral health care provider. However, often there are additional factors that impact health of the dentition. The oral cavity is composed of many tissue types including epithelium, connective tissue, blood vessels and lymphatic vessels. Uniquely, teeth are composed of pulp (blood vessels, nerves and collagen fibers), dentin, cementum and enamel, with the apical portions surrounded by bone. These tissues rely on both local and systemic factors for repair, regeneration and maintenance of health. For example, studies have shown the strong association between the use of fluoride and preventing dental caries where fluoride strengthens enamel through the formation of fluorohydroxyapatite (Buzalaf *et al.*, 2011). Conversely, mutations in certain genes can cause defects in enamel formation (Simmer and Hu, 2001), potentially increasing susceptibility to dental caries.

Vitamin D plays a pivotal role in calcium homeostasis and immune regulation. Calcium and vitamin D have a documented role in preventing demineralization, encouraging remineralization and establishing the strength of mineralized tissues (i.e. bone and teeth). Therefore, a plausible relationship exists between the health of the periodontium, which is directly influenced by the health of the surrounding soft and mineralized tissues, and the presence of vitamin D and calcium. Furthermore, vitamin D has been associated with the arrest and prevention of dental caries through its effects on enamel mineralization.

In this chapter, we will explore the link between vitamin D and key conditions that affect dental health. Specifically, the relationship between vitamin D and gingivitis, periodontitis and dental caries will be discussed. As vitamin D is a fat-soluble vitamin, there are potential negative effects in the oral cavity when vitamin D levels remain elevated. Accordingly, information regarding the toxicity of vitamin D in the oral cavity will also be discussed.

19.2 Vitamin D and gingivitis

19.2.1 Definition, nomenclature, and epidemiology

Diseases affecting the oral cavity typically impact the periodontium. The periodontium is defined as the surrounding and supporting structures of the tooth, and is broken down into two main categories, the gingiva and the attachment apparatus. The attachment apparatus consists of the periodontal ligament, cementum and alveolar bone. Cementum is an avascular calcified tissue, which comprises the outer portion of the root. The periodontal ligament is responsible for connecting the cementum (and therefore the root) to the surrounding alveolar bone (that portion of the maxilla and mandible forming the tooth socket). The gingiva is the soft tissue surrounding the tooth comprised of connective tissue covered by a layer of epithelium and its primary purpose is to protect the underlying tissues and referred to as the 'gums' in lay terminology. It aids in forming the gingival sulcus, which is a shallow 'V' shaped space bound by tooth structure on one side and epithelium on the other.

Gingivitis is an inflammatory condition confined to the soft tissues surrounding teeth that does not result in destruction of bone or the attachment apparatus (Armitage, 1999). The characteristic signs are redness and swelling of the gingiva, or gingiva that bleeds easily. Although gingival diseases can be related to a number of factors, the most common form is plaque-induced gingivitis. This gingivitis results from the presence of thin layer of bacteria and bacterial byproducts in a polysaccharide matrix, which is known as a dental biofilm (Page, 1986). Dental plaque is a biofilm that forms a soft deposit around teeth and other hard surfaces of the oral cavity. Plaque contains both organic and inorganic components but is primarily made up of microorganisms. These microorganisms are predominantly bacterial in origin and are roughly equal parts gram-positive and gram-negative. Some of the bacteria in the biofilm produce enzymes and tissue-destructive factors that can contribute to the breakdown of gingival tissues. However, the redness and swelling of the tissues seen in gingivitis is actually a manifestation of the body's immune response to the presence of these microbes. In the initial stages of gingivitis, increased vascular permeability and subsequent infiltration of neutrophils and lymphocytes occurs (Page *et al.*, 1997). Once permeability increases, microbes capable of producing products such as collagenase and endotoxin, may cause epithelial and connective tissue damage.

The etiology of gingivitis is multi-factorial but is most commonly due to the interaction of microorganisms within dental plaque and inflammatory cells of the gingival tissues. As with most inflammatory diseases, gingivitis can be modified by both local and systemic factors. Local factors that exacerbate gingival inflammation include the formation of calculus, a hard deposit that results from the mineralization of plaque. Systemic factors such as hormonal changes (puberty and pregnancy), medications (anticonvulsants, immunosuppressants, calcium channel blockers) and severe malnutrition may also exacerbate gingivitis.

Clinically gingivitis can be categorized as acute or chronic and localized or generalized. Diagnosis of gingivitis is typically made by elicitation of bleeding upon gentle probing of the gingival sulcus

(Amato *et al.*, 1986). Gingivitis is also demarcated by changes in color, texture and position of the gingiva. According to a national survey of the US population, approximately 54% of individuals age 13 years or older have gingivitis in at least one site (Surgeon Generals' Report, 2000). The same study found that gingivitis was most prevalent among younger and older age groups as compared to middle age groups. Gingivitis can be effectively treated by proper oral hygiene as well as regular dental prophylaxis.

19.2.2 Biological mechanisms of action of vitamin D in gingival tissue

Since bacteria and the immune response to these bacteria are the primary cause for diseases of the periodontium, this creates a unique environment for the role of the immune system in mediating conditions of the oral cavity. Evidence of the role of vitamin D in immunity is growing. VDR is a part of the superfamily of nuclear hormone receptors. VDRs are found in cells involved with calcium and bone metabolism, and immune regulation, and these receptors are present in cells of the oral cavity. When bound to the active form of vitamin D, $1,25(OH)_2D3$, VDRs bind to certain sequences in vitamin D-responsive genes and influence the rate of RNA polymerase II-mediated transcription (Mathieu and Adorini, 2002). $1,25(OH)_2D3$ binds to VDRs on T cells and antigen-presenting cells, thereby modulating the immune response. Because VDRs function based on binding to the active form of vitamin D, the enzyme responsible for the conversion of 25(OH)D into $1,25(OH)_2D3$ is crucial. In an animal study, the 25(OH)D-[1(OH)ase] enzyme was ablated in mice, resulting in hypocalcemia, secondary hyperparathyroidism and a reduction in $CD4^+$ and $CD8^+$ T cell populations (Panda *et al.*, 2001).

The anti-inflammatory effects of vitamin D are well-supported through *invitro* human studies. The biologically active form, $1,25(OH)_2D3$ has been shown to decrease antigen-induced T cell proliferation through the inhibition of IL-2 (Bhalla *et al.*, 1984), as well as inhibit the production of IFN- by T-lymphocytes (Rigby *et al.*, 1987). Furthermore, several studies have documented the role of $1,25(OH)_2D3$ in inhibiting the differentiation, maturation and proliferation of antigen presenting cells (Berer *et al.*, 2000; Penna and Adorini, 2000; Piemonti *et al.*, 2000). 1,25-dihydroxyvitamin D has also been shown to impact innate immunity, leading to the destruction of infectious agents (i.e. *Mycobacterium tuberculosis*). When macrophages or monocytes are stimulated by an LPS or infectious agent, the expression of the VDR and the 25(OH)D-[1(OH)ase] enzyme are up-regulated; this in turn increases the production of 1,25-dihydroxyvitamin D, which increases the expression of cathelicidin (Holick, 2007). Cathelicidins are peptides capable of promoting innate immunity and promoting the destruction of infectious agents. They have documented antimicrobial activity, effective against both gram positive and gram negative bacteria (Zanetti *et al.*, 2004). They have also been shown to impact the activity of antigen presenting cells (Bowdish *et al.*, 2006). Thus, vitamin D may play a critical role in influencing the effect of bacteria on periodontal destruction through mediating the immune response to bacteria as well as antimicrobial effects.

19.2.3 Review of studies correlating vitamin D and gingivitis

In a recent study, McMahon *et al.* (2011) described the impact of vitamin D on gingival epithelial cells and their defense against the well-established periodontal pathogen *Aggregatibacter actinomycetemcomitans* (McMahon *et al.*, 2011). Exposure of epithelial cells to the active form of vitamin D, $1,25(OH)_2D3$, induced the expression of the human cathelicidin LL-37 and also the innate immune regulator triggering receptor on myeloid cells (triggering receptor on myeloid cells), which has also been shown to impact the regulation of innate immune response. These gingival epithelial cells were shown to have increased antibacterial activity, measured through their increased ability to destroy *A. actinomycetemcomitans*. These findings support the notion of the effect of vitamin D on immune regulation and innate immunity in the oral cavity.

Even with the evidence of the role of vitamin D and its impact on modulators of inflammation and immunity, information regarding the role of the anti-inflammatory effects of vitamin D in humans is limited. A study by Dietrich *et al.* (2005) examined data from the 3rd NHANES data set and made associations between patients' serum vitamin D levels and chronic gingivitis (Dietrich *et al.*, 2005). They found a strong negative association between the serum level of vitamin D and chronic gingivitis, as indicated by BOP. Subjects in the top quintile of serum vitamin D levels had 20% lower odds of BOP; they also found that increasing the vitamin D levels by 30 nmol/l was associated with a 10% lower odds for BOP. Because of the known function of the immune response in the formation of gingivitis and the developing relationship between vitamin D and the immune response, there is reason to assume a possible association between vitamin D and gingivitis. Findings such as those made by Dietrich substantiate further investigation into this relationship.

19.3 Vitamin D and periodontitis

19.3.1 Definition, nomenclature, and epidemiology

Periodontitis is defined as 'an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in the progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both'. Unlike gingivitis where the inflammatory process affects the gingival tissues only, periodontitis results in destruction of bone and connective tissue attachment around the tooth (Page, 1986). The result of attachment loss is typically the formation of a periodontal pocket, or pathological deepening of the gingival sulcus, which serves as a reservoir for pathogenic bacteria. Periodontitis is classified into three main categories based on its etiology, progression and response to therapy and includes: chronic periodontitis, aggressive periodontitis, and periodontitis as a manifestation of systemic disease (Armitage, 1999). Diagnosis of periodontal disease is based on an evaluation of dental radiographs demonstrating bone loss, probing of the periodontal pocket to measure attachment loss as well as the presence of bleeding and numerous other clinical parameters (Page, 1986).

Chronic periodontitis is the most common form of periodontal disease and is typically seen in adults older than 35 years of age (Papapanou, 1996). The primary etiology of this condition is the host immune response to the presence of bacterial plaque. Characteristically, chronic periodontitis causes a slow to moderate rate of destruction but may have rapid bursts of activity in the presence of local, systemic or environmental factors. Disease severity is generally based on the length of time the periodontal tissues have been challenged by inflammation. Because dental plaque is the main cause of chronic periodontitis, any plaque retentive factors such as calculus (calcified plaque), will aid in the progression of the disease. Calculus, which firmly attaches to the root surface, gives bacterial plaque an ideal place to harbor and grow. Therapeutic measures to control this condition are aimed at removal of calculus and control of periodontal inflammation.

Aggressive periodontitis differs from chronic periodontitis in that the rate of bone breakdown and attachment loss is typically rapid. Furthermore, the amount of plaque and calculus is minor when compared to the amount of destruction present. Despite the presence of deep periodontal pockets, the amount of inflammation present is generally minimal. Interestingly, aggressive periodontal disease is typically found in individuals under the age of 30 and may even show a pubertal onset but only affects a very small segment of the population (Papapanou, 1996). A genetic component appears to be present in aggressive periodontitis based on a familial aggregation of the disease. Some, but not all individuals with this condition may show abnormalities in phagocyte function or a hyper responsiveness of macrophages pointing to an abnormal immune response.

Periodontitis as a manifestation of systemic disease can be found in individuals with certain hematologic and genetic conditions. Leukemias, Down syndrome and leukocyte adhesion deficiencies are among some of the conditions that lead to periodontal destruction. In general, it is believed that a disruption in the host defense mechanisms is responsible for the disease progression observed.

19.3.2 Review of studies correlating vitamin D and periodontitis

Because of the well-established link of vitamin D and its relationship to calcium homeostasis and regulation in bones, as well as its role in host immunity, there is biological plausibility to assume a relationship between vitamin D and periodontal health. Table 19.1 summarizes pertinent studies evaluating this relationship.

In animal models, vitamin D deficiency has been shown to increase osteoclast activity and bone resorption. Anderson *et al.* (2011) showed that in rats, serum vitamin D levels directly impacted bone mineralization. The rats were given a diet of 0.4% calcium and different levels of vitamin D3, which produced serum levels ranging between 10 and 115 nmol/l. There was a positive correlation between the trabecular bone mineral volume (measured in the distal femoral metaphysis) and the circulating 25(OH)D levels (r^2=0.42, P<0.01). There was an observed increase in the ratio of receptor activator of NFκB ligand:OPG mRNA for animals with low serum vitamin D levels. The receptor activator of NFκB ligand is a mediator of osteoclastogenesis and OPG is a receptor decoy antagonist. In an unrelated study, periodontal findings were evaluated in sheep that had

Table 19.1. Studies relating vitamin D with gingivitis and periodontitis.

Authors	Subject demographic	Dental outcome measurement	Systemic outcome measurement	Type of study	Results
Dietrich *et al.*, 2005	6,700 adults aged 13 to >90 years	Gingivitis measured by bleeding on probing	Serum concentration 25(OH)D	Cross-sectional	Subjects in the highest 25(OH)D quintile were 20% less likely to have bleeding on probing than the lowest quintile.
Krall *et al.*, 2001	145 healthy adults aged 65 yrs and over	Tooth loss	Supplementation with calcium (500 mg/day) and vitamin D (700 IU/day)	Randomized controlled trial	Fewer subjects (13% vs. 27%) taking supplements lost teeth during the trial period and during the two year follow up period (40% vs. 59%) than those not taking supplements.
Dietrich *et al.*, 2004	11,202 adults ≥20 yrs	Clinical attachment loss	Serum concentration 25(OH)D	Cross-sectional	Inverse association between serum 25(OH)D levels and attachment loss.
Miley *et al.*, 2009; Garcia *et al.*, 2010	51 postmenopausal women and men aged 50-80	Probing depths, sites with bleeding on probing, clinical attachment loss, alveolar bone crest height	Supplementation with calcium (≥1000 mg/day) and vitamin D (≥400 IU/day)	Cross-sectional/ longitudinal	Subjects in the supplementation group had shallower probing depths, fewer sites with bleeding on probing, less attachment loss and less alveolar bone crest height loss, compared with those not taking supplementation.
Bashutski *et al.*, 2011	40 adults (aged 30-65)	Probing depths and clinical attachment loss	Serum 25(OH)D	Randomized controlled trial	Vitamin D sufficient subjects (≥20 ng/ml) showed more CAL gain (0.92 vs. -0.43 mm) and greater pocket depth reduction (1.83 mm vs. 0.43 mm) than vitamin D deficient patients (16-19 ng/ml)
Chen *et al.*, 2011	Asian and Caucasian adults	CP or AP	VDR polymorphisms: *TaqI*, *BsmI*, *FokI*, and *ApaI*	Meta-analysis	Significant association between the VDR *TaqI* variants and CP in Asians *FokI* allele is a risk factor for AP
Deng *et al.*, 2011	Asian and Caucasian adults	CP or AP	VDR polymorphisms: *TaqI*, *BsmI*, *FokI*, and *ApaI*	Meta-analysis	Significant association between *TaqI* variant and CP Among Asian CP patients, lower frequencies of the *BsmI* variant and higher frequencies of the *ApaI* variants

been given a calcium and vitamin D-restricted diet, ovariectomy, and glucocorticoid injections, and compared with sheep without these alterations (Dvorak *et al.*, 2009). The authors reported that sheep in the test group had more gingival recession than did those of the control group. Of note, however, is the age of the test group (mean 3.8±0.9 years) was different than the control group (mean 7.5±1.0 years), as age can influence degenerative changes of the periodontium. Furthermore, the authors admit that they could not implicate which single parameter influenced changes in the periodontium.

Although limited evidence of vitamin D and periodontal disease exists in animal studies, there has been growing work examining this relationship in humans. Patients with vitamin D-dependent rickets, who have a hereditary defect in vitamin D metabolism, frequently present with chronic periodontal disease (Zambrano *et al.*, 2003). In a 2001 study, Krall reported a decreased incidence of tooth loss in elderly patients taking calcium and vitamin D supplementation versus those who did not (13% vs. 27%) (Krall *et al.*, 2001). These effects lasted even after the end of supplementation for up to two years, as only 40% of subjects supplementing 1000 mg calcium lost one or more teeth, compared to nearly 60% of subjects who took less. Although the results of this study pointed to the impact of vitamin D and calcium supplementation on tooth loss, specific parameters of periodontitis were not measured and the tooth loss cannot be attributed to periodontal disease alone. However, in a 2004 report, data from the third National Health and Nutrition Examination Survey were used to evaluate the association between serum 25(OH)D levels and indicators of periodontal disease (Dietrich *et al.*, 2004). It was shown that for persons >50 years old, there was a statistically significant, inverse association with attachment loss and serum vitamin D levels. When comparing persons from the lowest quintile of serum vitamin D with those from the highest quintile, there was 0.39 mm greater attachment loss for men and 0.26 mm greater attachment loss for women. Similarly, among patients receiving periodontal therapy, those with the highest serum vitamin D level show significantly less bleeding sites and lower mean pocket depth and clinical attachment (Teles *et al.*, 2011). In a cohort of patients undergoing periodontal maintenance therapy, although marginally significant, patients who took ≥400 IU/day of vitamin D and ≥1000 mg/day of calcium had shallower probing depths, less attachment loss, fewer bleeding sites and less alveolar crest height loss for up to one year (Garcia *et al.*, 2010; Miley *et al.*, 2009). Though these studies did not directly measure serum 25(OH)D levels, the effects of supplementation are noteworthy and revealed the need for further investigation into this area.

Only one study has provided insight into the impact of serum vitamin D levels and response to periodontal therapy in patients with advanced periodontal disease. A post-hoc analysis of a study involving the anabolic peptide teraparatide showed a positive correlation between serum vitamin D levels and periodontal parameters (Bashutski *et al.*, 2011). Forty subjects with severe chronic periodontitis planned for periodontal surgery were given teriparatide or placebo in conjunction with daily vitamin D and calcium supplements. Both clinical and radiographic parameters were measured over the course of 1 year and serum vitamin D levels were measured at baseline, 6 weeks and 6 months post surgery. The authors reported that placebo patients with vitamin D deficiency (defined as 16-19 ng/ml serum 25(OH)D) at the time of surgery had significantly less

clinical attachment level gain and pocket depth reduction than vitamin D sufficient patients at 12 months. Interestingly, even with growing evidence of the potential benefits of vitamin D and periodontal health, only 7% of adults enrolled in a periodontal maintenance program report taking the US Food and Nutrition Board's recommended amount of supplementation (Dixon *et al.*, 2009). This suggests that patients at high risk for periodontitis are also receiving inadequate amounts of vitamin D.

The role of vitamin D in periodontal health is gaining popularity, with increased interest into the precise nature of the association. This relationship, however, may not be limited solely to supplementation and circulating levels of serum 25(OH)D. There is evidence to suggest a prominent role of the VDR and its impact on periodontitis. Mutations in the VDR gene have been linked to diseases such as cancer, primary hypoparathyroidism, and renal failure (Valdivielso and Fernandez, 2006). Accordingly, VDR polymorphisms have been associated with both chronic and aggressive periodontitis (Meng *et al.*, 2007; Yoshie *et al.*, 2007). Four VDR polymorphisms have been identified in association with periodontal diseases: VDR *TaqI*, VDR *BsmI*, VDR *FokI*, and VDR *ApaI*. A meta-analysis by Chen *et al.* (2011) found a significant association between the *TaqI* variants and chronic periodontitis in Asians and found that the *FokI* allele is a risk factor for aggressive periodontitis. Deng *et al.* (2011) also conducted a meta-analysis examining the same four polymorphisms. Like the previous study, these authors found a similar association between the *TaqI* variants and chronic periodontitis; however, they also reported that among Asian chronic periodontitis patients, lower frequencies of the *BsmI* variant and higher frequencies of the *ApaI* variants were found.

Such findings highlight the interaction between vitamin D, its receptor, and maintenance of periodontal health. Vitamin D contributes directly to bone strength and plays a role in host immunity, both of which can alter the pathogenesis of periodontitis.

19.4 Vitamin D and dental caries

19.4.1 Definition, nomenclature, and epidemiology

As reported by the 2000 US Surgeon General report, dental caries is the single most common chronic childhood disease. Dental caries is five times more common than asthma and seven times more common than hay fever. Reports from the NHANES from 1988-1994 and from 1999-2004 suggest that the prevalence of dental caries is increasing, despite improvements in a number of other areas and factors such as sealants, tooth retention, periodontal health, and edentulism (Dye *et al.*, 2007).

Early studies looking at the properties of vitamin D supplementation in children were conducted as early as 1924 where vitamin D was proposed to play a critical role in tooth development along with the theory that properly developed teeth are less susceptible to caries, which was a point of contention at the time with some dental authorities (Mellanby, 1924). Mellanby and Pattison

(1928) evaluated the use of dietary supplements, and determined that the addition of vitamin D prevented the initiation of new carious foci, limited the spread of caries and arrested active caries. McBeath (1934) evaluated 425 institutionalized males and females, ages eight to fourteen, and found that those receiving increased levels of vitamin D in evaporated milk and dairy milk, compared to positive and negative controls, had fewer dental caries. This group concluded that vitamin D is an important factor in the nutritional control of dental caries.

Studies have analyzed the regional geographic differences between the amount of average hours of sunlight and prevalence of caries (McBeath, 1934). In a study of 94,337 Caucasian boys ages 12-14, East (1939) found an inverse relationship between the hours of sunlight exposure in areas of residence and caries rate.

19.4.2 Impact on vitamin D on teeth

The ability of vitamin D to affect the calcification of tooth enamel of permanent teeth, the rate of tooth eruption, and the position of teeth was suggested as early as 1918 (Mellanby, 1918). Physical effects of vitamin D deficiency in teeth include dysplastic enamel in the form of enamel hypoplasia (Liu *et al.*, 2009; Papagerakis *et al.*, 1999) and is commonly associated with vitamin D-dependent rickets type I, hereditary vitamin D-dependecy rickets, hypoparathryoidism, and other developmental disorders (Nikiforuk and Fraser, 1981).

Structurally, tooth-specific matrix proteins are differentially regulated by vitamin D3 during tooth development. Papagerakis *et al.* (1999) found that vitamin D3 up-regulates the transcription of amelogenin, and presumably plays a role in the genetic and hormonal control of enamel formation as seen in rats (Liu *et al.*, 2009). Findings in vitamin D deficient animals include enamel defects found in the main enamel prism proteins as well as alterations in dentin formation with respect to shape, thickness, and composition (Papagerakis *et al.*, 2002).

The effects of vitamin D metabolism are directly linked to the physiologic levels of calcium and phosphorus available extracellularly (Christakos *et al.*, 2010; Liu *et al.*, 2009). Lui *et al.* (2009) suggest that defects in teeth and mandibles during mineralization induced by vitamin D3 deficiency are similar to long bone defects as seen in 1α-hydroxylase knockout mice, which represents the vitamin D-dependent rickets type I loss of function mutation. Similarly, Krall *et al.* (2001) found that supplements of calcium and vitamin D in an elderly population of men and women contribute to a reduced the risk of tooth loss, a reduction of moderate bone loss, and nonvertebral fracture risk.

19.4.3 Review of studies correlating vitamin D and caries

Vitamin D and its relationship to dental caries was initially presented in the 1920s and 1930s (East, 1939; McBeath, 1934; Mellanby and Pattison, 1928) and has been continually studied to the present day. Table 19.2 summarizes several of the studies between vitamin D and dental caries, many of which support the benefits of nutritional supplementation with vitamin D to improve oral

health. Mellanby and Pattison (Mellanby and Pattison, 1928) stated in multiple publications that diets high in vitamin D-containing foods caused a 'hardening' effect on existing carious lesions and acted by suppressing the carious process. While there are limitations of their study in regards to how effectively the amount of available vitamin D from the dietary sources could be measured, these studies support the ability of carious lesions to be reversed through remineralization in the presence of adequate vitamin D. In a similar study, McBeath (1934) also applied dietary changes by increasing vitamin D intake through increased milk consumption. In addition, he added a group who received ultra-violet lamp treatments to further increase the amount of vitamin D the patients received. He then examined each individual and recorded the total number of carious surfaces as a percentage of the number of surfaces in the mouth over the course of a year and found an inverse correlation between vitamin D intake and the presence of caries. Pacey *et al.* (2010), through a cross-sectional health survey found a protective effect associated with milk intake, while sugar intake had a deleterious association in respect to reported caries experience. Schroth *et al.* (2005) considered maternal vitamin D supplementation with respect to primary tooth eruption, mean number of teeth with enamel hypoplasia and decayed, extracted, and filled teeth. No association was found with vitamin D supplementation and fewer decayed teeth; however, the eruption of primary teeth was found to be earlier in mothers who received vitamin D supplementation. Krall *et al.* (2001) evaluated tooth loss in the elderly using a longitudinal study and found that calcium and vitamin D supplementation prevented tooth loss. Possibly one of the longest longitudinal studies on the effects of vitamin D on caries was conducted over a 25-year period and evaluated calcium and phosphate levels in individuals with vitamin D deficiency over time (Nikiforuk and Fraser, 1981). Disorders that were monitored in this study included hereditary vitamin D-dependent rickets, X-linked hypophosphatemic rickets, permanent hypoparathyroidism, transient neonatal hypoparathroidism and pseudohypoparathyroidism. This study observed the prevalence of enamel hypoplasia in both the primary and permanent dentition as well as the presence of dental lesions and found a positive association between hypocalcemia and enamel hypoplasia. No relationship was observed between hypophosphatemia and enamel lesions. Environmental factors such as sunlight exposure and regional location have also been evaluated for their role in dental caries, through indirect changes in vitamin D levels. Many studies have found that caries incidence is inversely related with annual sunlight exposure (East, 1939; Fraser and Nikiforuk, 1982; Krall *et al.*, 2001).

19.5 Vitamin D toxicity in the oral cavity

While a deficiency in vitamin D may prevent calcification and compromise immunity, high levels of vitamin D may also be detrimental to oral tissues through promoting extensive bone resorption, ectopic calcifications and alterations in dental tooth morphology. A case report of a young girl affected by hypervitaminosis D showed enamel hypoplasia and focal pulp calcifications in the permanent teeth (Giunta, 1998). The effects of hypervitaminosis D on tooth structure may also include enamel hypoplasia, hypermineralization of enamel matrix, defective dentine formation, excessive hypercementosis and calcification of pulpal and periodontal tissues (Cagnone, 1970). However, this hypermineralization may have protective effects against caries as well. One study

Table 19.2. Studies relating vitamin D with caries.

Authors	Subject demographic	Dental outcome measurement	Systemic outcome measurement	Type of study	Results
Mellanby and Pattison, 1928	21 children	Caries	N/A	Cross-sectional	Carious process could be inhibited by increasing the intake of fat-soluble vitamins
Mellanby and Pattison, 1932	22 children	Caries	N/A	Cross-sectional	Vitamin D and calcium rich diets that lack cereals has a greater inhibitory and curative effects on dental caries
McBeath, 1934	425 children	Total number of carious tooth surfaces	N/A	Cross-sectional	Correlation of degree of control of caries with a quantitative dose of vitamin D
East, 1939	94,337 boys, aged 12-14	Mean caries incidence per 100 children	Mean annual amounts of sunshine in hours	Cross-sectional	Mean caries incidence varies inversely with the hours of sunshine present in the area of residence
Nikiforuk and Fraser, 1981	56 children with vitamin D abnormalities	Enamel hypoplasia	Hypocalcemia	Longitudinal	Enamel hypoplasia occurred only in children who had hypocalcemia
Krall *et al.*, 2001	145 healthy adults (aged 65 or older)	Tooth loss	N/A	Longitudinal	Intake levels of calcium and vitamin D used for preventing osteoporosis are beneficial to tooth retention
Schroth *et al.*, 2005	98 children	DEFT	N/A	Cross-sectional dental examination	Mean DEFT for study group was high and 50% had enamel defects
Pacey *et al.*, 2010	388 Nunavut Inuit children	RCE	Nutritional supplements, food questionnaire, dietary recalls	Cross-sectional health survey	High prevalence of RCE. Milk intake showed protective associations. Sugar intake showed deleterious associations with RCE

reported that indirect effects of high levels of vitamin D, as assessed through ultraviolet radiation exposure, reduced caries rates. Interestingly, there was no adverse effect on teeth at higher levels of exposure, although this would not necessarily relate to toxic levels of vitamin D (Grant, 2011).

Hypervitaminosis D also affects the tissues surrounding teeth including alveolar bone, connective tissue, and epithelium. The effects of high levels of vitamin D on several tissue types were compared to a control group who received normal levels using an animal model (Haschek *et al.*, 1978). In this porcine study, 825,000 IU/kg of vitamin D had detrimental effects to both bone and soft tissue within 2 weeks of administration. Notably, osteonecrosis, osteocytic osteolysis, and epithelial cell degeneration occurred. Although this study did not specifically evaluate oral tissues, both bone and epithelial integrity is critical to prevent many types of oral disease. An animal study evaluated the effects of 5,000 IU vitamin D/10 g body weight on the developing bones and teeth of rats (Hammarstrom *et al.*, 1973). Within days, acid phosphatase activity increased in the alveolar bone regions, but did not increase in tooth enamel. In addition, ectopic calcification occurred near erupting teeth and in the connective tissues surrounding teeth, which ultimately altered the developing tooth morphology. Consequently, the effects of excessive levels of vitamin D in the oral cavity include increased alveolar bone resorption, loss of epithelial and connective tissue integrity, increased enamel mineralization, and tooth anomalies. While an insufficient number of studies have been conducted in humans, this information suggests that patients with hypervitaminosis D may be at increased risk for chronic periodontitis, altered tooth eruption, and decreased caries risk.

19.6 Summary and conclusions

Vitamin D is necessary for mineralization and immune support, both of which affect conditions of the oral cavity including gingivitis, periodontitis, and caries. Figure 19.1 summarizes the proposed effects of vitamin D deficiency on teeth and the periodontium. Vitamin D has anti-inflammatory and anti-bacterial roles in the oral cavity. Its anti-inflammatory effects are a result of many different interactions including activation of both acquired and innate immunity though upregulation of T-cells and antigen-presenting cells, as well as a feedback loop of increased VDR activation when monocytes and macrophages are infected by bacteria. The presence of vitamin D also provides indirect antimicrobial effects through increasing cathelicidin levels, which destroy both gram negative and gram positive bacteria. As a result, vitamin D deficiency may compromise immunity and allow bacterial growth within the oral cavity, ultimately increasing local inflammation and the development of gingivitis.

Vitamin D is also necessary for mineralization of dental tissues including alveolar bone, enamel, and dentin. Inadequate mineralization of these tissues as a result of vitamin D deficiency can contribute to decreased integrity of these structures, placing them at higher risk for disease. In the presence of inflammation and pathogenic bacteria, which is also exacerbated by vitamin D deficiency, oral conditions such as periodontitis and caries may be at higher risk of development.

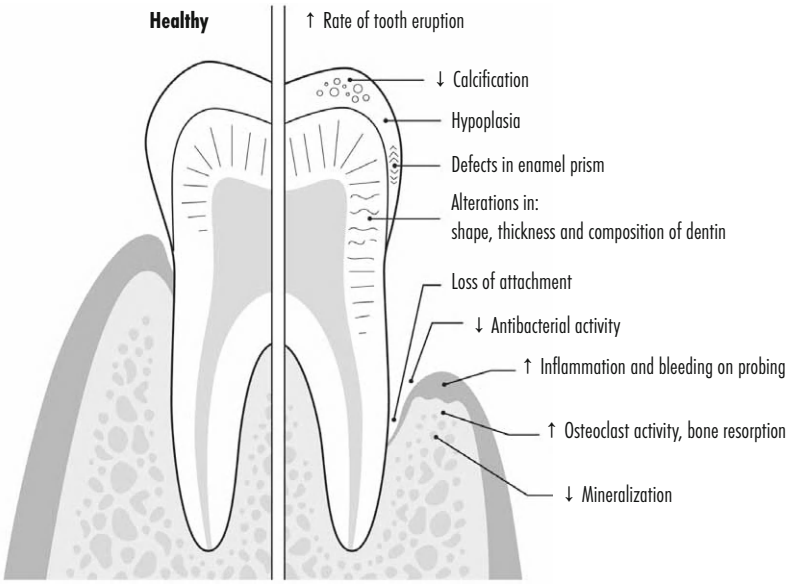

Figure 19.1. Effects of vitamin D deficiency on the tooth. The left side of the figure shows a healthy tooth and surrounding periodontium. The right side shows proposed effects of vitamin D deficiency on the tooth and surrounding periodontium.

While most evidence shows an association between vitamin D deficiency and conditions of the oral cavity, it is important to remember that oral diseases are often multifactorial. Evidence linking vitamin D deficiency with increased risk for oral disease requires more investigation to show causality and determine the magnitude of the contribution. Vitamin D is involved in many vital aspects of mineralization, immunity, and tissue integrity that suggest a key role in preventing oral disease.

References

Amato, R., Caton, J., Polson, A. and Espeland, M., 1986. Interproximal gingival inflammation related to the conversion of a bleeding to a nonbleeding state. Journal of Periodontology 57, 63-68.

Anderson, P.H., Atkins, G.J., Turner, A.G., Kogawa, M., Findlay, D.M. and Morris, H.A., 2011. Vitamin D metabolism within bone cells: effects on bone structure and strength. Molecular and Cellular Endocrinology 347, 42-47.

Anonymous, 2000. Surgeon General's Report on Oral Health. Available at: http://www.surgeongeneral.gov/library/oralhealth/.

Armitage, G.C., 1999. Development of a classification system for periodontal diseases and conditions. Annals of Periodontology 4, 1-6.

Bashutski, J.D., Eber, R.M., Kinney, J.S., Benavides, E., Maitra, S., Braun, T.M., Giannobile, W.V. and McCauley, L.K., 2011. The Impact of Vitamin D Status on Periodontal Surgery Outcomes. Journal of Dental Research 90, 1007-1012.

Berer, A., Stockl, J., Majdic, O., Wagner, T., Kollars, M., Lechner, K., Geissler, K. and Oehler, L., 2000. 1,25-Dihydroxyvitamin D(3) inhibits dendritic cell differentiation and maturation *in vitro*. Experimental Hematology 28, 575-583.

Bhalla, A.K., Amento, E.P., Serog, B. and Glimcher, L.H., 1984. 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. Journal of Immunology 133, 1748-1754.

Bowdish, D.M., Davidson, D.J. and Hancock, R.E., 2006. Immunomodulatory properties of defensins and cathelicidins. Current Topics in Microbiology and Immunology 306, 27-66.

Buzalaf, M.A., Pessan, J.P., Honorio, H.M. and ten Cate, J.M., 2011. Mechanisms of action of fluoride for caries control. Monographs in Oral Science 22, 97-114.

Cagnone, L., 1970. Vitamin Disorders. In: R. Gorlin and H. Goldman (eds.), Thoma's Oral Pathology. Mosby, St. Louis, USA, pp. 610-618.

Chen, L., Li, H., Zhang, P. and Wang, S., 2011. Association Between Vitamin D Receptor Polymorphisms and Periodontitis: A Meta-Analysis. Journal of Periodontology.

Deng, H., Liu, F., Pan, Y., Jin, X., Wang, H. and Cao, J., 2011. BsmI, TaqI, ApaI, and FokI polymorphisms in the vitamin D receptor gene and periodontitis: a meta-analysis of 15 studies including 1338 cases and 1302 controls. Journal of Clinical Periodontology 38, 199-207.

Dietrich, T., Joshipura, K.J., Dawson-Hughes, B. and Bischoff-Ferrari, H.A., 2004. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. American Journal of Clinical Nutrition 80, 108-113.

Dietrich, T., Nunn, M., Dawson-Hughes, B. and Bischoff-Ferrari, H.A., 2005. Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation. American Journal of Clinical Nutrition 82, 575-580.

Dixon, D., Hildebolt, C.F., Miley, D.D., Garcia, M.N., Pilgram, T.K., Couture, R., Anderson Spearie, C. and Civitelli, R., 2009. Calcium and vitamin D use among adults in periodontal disease maintenance programmes. British Dental Journal 206, 627-631; discussion 617.

Dvorak, G., Reich, K., Tangl, S., Lill, C.A., Gottschalk-Baron, M., Watzek, G., Gruber, R. and Haas, R., 2009. Periodontal histomorphometry and status of aged sheep subjected to ovariectomy, malnutrition and glucocorticoid application. Archives of Oral Biology 54, 857-863.

Dye, B.A., Tan, S., Smith, V., Lewis, B.G., Barker, L.K., Thornton-Evans, G., Eke, P.I., Beltran-Aguilar, E.D., Horowitz, A.M. and Li, C.H., 2007. Trends in oral health status: United States, 1988-1994 and 1999-2004. Vital and Health Statistics. Series 11: Data from the National Health Survey, 1-92.

East, B.R., 1939. Mean Annual Hours of Sunshine and the Incidence of Dental Caries. American Journal of Public Health and the Nations Health 29, 777-780.

Fraser, D. and Nikiforuk, G., 1982. The etiology of enamel hypoplasia in children – a unifying concept. Journal of the International Association of Dentistry for Children 13, 1-11.

Garcia, M.N., Hildebolt, C.F., Miley, D.D., Dixon, D.A., Couture, R.A., Anderson Spearie, C.L., Langenwalter, E.M., Shannon, W.D., Deych, E., Mueller, C. and Civitelli, R., 2010. One-year Effects of Vitamin D and Calcium Supplementation on Chronic Periodontitis. Journal of Periodontology 82, 25-32.

Giunta, J.L., 1998. Dental changes in hypervitaminosis D. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 85, 410-413.

Grant, W.B., 2011. A review of the role of solar ultraviolet-B irradiance and vitamin D in reducing risk of dental caries. Dermato-Endocrinology 3, 193-198.

Hammarstrom, L.E., Ramp, W.K. and Toverud, S.U., 1973. Histochemistry of various acid phosphatases in developing bones and teeth in hypervitaminosis D in the rat. Archives of Oral Biology 18, 109-115.

Haschek, W.M., Krook, L., Kallfelz, F.A. and Pond, W.G., 1978. Vitamin D toxicity. Initial site and mode of action. Cornell Veterinarian 68, 324-364.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Krall, E.A., Wehler, C., Garcia, R.I., Harris, S.S. and Dawson-Hughes, B., 2001. Calcium and vitamin D supplements reduce tooth loss in the elderly. American Journal of Medicine 111, 452-456.

Liu, H., Guo, J., Wang, L., Chen, N., Karaplis, A., Goltzman, D. and Miao, D., 2009. Distinctive anabolic roles of 1,25-dihydroxyvitamin D(3) and parathyroid hormone in teeth and mandible versus long bones. Journal of Endocrinology 203, 203-213.

Mathieu, C. and Adorini, L., 2002. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. Trends in Molecular Medicine 8, 174-179.

McBeath, E.C., 1934. Vitamin D Studies, 1933-1934. American Journal of Public Health and the Nations Health 24, 1028-1030.

McMahon, L., Schwartz, K., Yilmaz, O., Brown, E., Ryan, L.K. and Diamond, G., 2011. Vitamin D-mediated induction of innate immunity in gingival epithelial cells. Infection and Immunity 79, 2250-2256.

Mellanby, E., 1924. A British Medical Association Lecture on Deficiency Diseases, with special reference to Rickets. Br Med J. 24, 895-900.

Mellanby, M., 1918. An Experimental Study of the Influence of Diet on Tooth Formation. Lancet 2, 767-770.

Mellanby, M., Pattison, C.L., 1928. The Action of Vitamin D in Preventing the Spread and Promoting the Arrest of Caries in Children. Br Med J. 15, 1079-1082.

Meng, H., Xu, L., Li, Q., Han, J. and Zhao, Y., 2007. Determinants of host susceptibility in aggressive periodontitis. Periodontology 2000 43, 133-159.

Miley, D.D., Garcia, M.N., Hildebolt, C.F., Shannon, W.D., Couture, R.A., Anderson Spearie, C.L., Dixon, D.A., Langenwalter, E.M., Mueller, C. and Civitelli, R., 2009. Cross-sectional study of vitamin D and calcium supplementation effects on chronic periodontitis. Journal of Periodontology 80, 1433-1439.

Nikiforuk, G. and Fraser, D., 1981. The etiology of enamel hypoplasia: a unifying concept. Journal of Pediatrics 98, 888-893.

Pacey, A., Nancarrow, T. and Egeland, G.M., 2010. Prevalence and risk factors for parental-reported oral health of Inuit preschoolers: Nunavut Inuit Child Health Survey, 2007-2008. Rural and Remote Health 10, 1368.

Page, R.C., 1986. Gingivitis. Journal of Clinical Periodontology 13, 345-359.

Page, R.C., Offenbacher, S., Schroeder, H.E., Seymour, G.J. and Kornman, K.S., 1997. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontology 2000 14, 216-248.

Panda, D.K., Miao, D., Tremblay, M.L., Sirois, J., Farookhi, R., Hendy, G.N. and Goltzman, D., 2001. Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci U S A 98, 7498-7503.

Papagerakis, P., Hotton, D., Lezot, F., Brookes, S., Bonass, W., Robinson, C., Forest, N. and Berdal, A., 1999. Evidence for regulation of amelogenin gene expression by 1,25-dihydroxyvitamin D(3) *in vivo*. Journal of Cellular Biochemistry 76, 194-205.

Papagerakis, P., MacDougall, M. and Berdal, A., 2002. Differential epithelial and mesenchymal regulation of tooth-specific matrix proteins expression by 1,25-dihydroxyvitamin D3 *in vivo*. Connective Tissue Research 43, 372-375.

Papapanou, P.N., 1996. Periodontal diseases: epidemiology. Annals of Periodontology 1, 1-36.

Penna, G. and Adorini, L., 2000. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. Journal of Immunology 164, 2405-2411.

Piemonti, L., Monti, P., Sironi, M., Fraticelli, P., Leone, B.E., Dal Cin, E., Allavena, P. and Di Carlo, V., 2000. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. Journal of Immunology 164, 4443-4451.

Rigby, W.F., Yirinec, B., Oldershaw, R.L. and Fanger, M.W., 1987. Comparison of the effects of 1,25-dihydroxyvitamin D3 on T lymphocyte subpopulations. European Journal of Immunology 17, 563-566.

Schroth, R.J., Smith, P.J., Whalen, J.C., Lekic, C. and Moffatt, M.E., 2005. Prevalence of caries among preschool-aged children in a northern Manitoba community. Journal of the Canadian Dental Association. Journal de L'Association Dentaire Canadienne 71, 27.

Simmer, J.P. and Hu, J.C., 2001. Dental enamel formation and its impact on clinical dentistry. Journal of Dental Education 65, 896-905.

Teles, F.R., Teles, R.P., Martin, L., Socransky, S.S. and Haffajee, A.D., 2011. Relationships Among IL-6, TNF-alpha, Adipokines, Vitamin D and Chronic Periodontitis. Journal of Periodontology 83, 1183-1191.

Valdivielso, J.M. and Fernandez, E., 2006. Vitamin D receptor polymorphisms and diseases. Clinica Chimica Acta 371, 1-12.

Yoshie, H., Tai, H., Kobayashi, T., Oda-Gou, E., Nomura, Y., Numabe, Y., Ito, K., Kurihara, H. and Kamoi, K., 2007. Salivary enzyme levels after scaling and interleukin-1 genotypes in Japanese patients with chronic periodontitis. Journal of Periodontology 78, 498-503.

Zambrano, M., Nikitakis, N.G., Sanchez-Quevedo, M.C., Sauk, J.J., Sedano, H. and Rivera, H., 2003. Oral and dental manifestations of vitamin D-dependent rickets type I: report of a pediatric case. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 95, 705-709.

Zanetti, M., Braghetta, P., Sabatelli, P., Mura, I., Doliana, R., Colombatti, A., Volpin, D., Bonaldo, P. and Bressan, G.M., 2004. EMILIN-1 deficiency induces elastogenesis and vascular cell defects. Molecular and Cellular Biology 24, 638-650.

Key facts

- Vitamin D is mainly produced by ultraviolet B radiation in the skin, and has to be hydroxylated in liver and kidney to its active form 1,25-dihydroxyvitamin D. After ligation to vitamin D receptor, it modulates gene transcription in a wide range of cells.
- These effects have been shown in the immune system, inducing a shift from a pro-inflammatory Th1/Th17 response to an anti-inflammatory Th2/Treg profile.
- Vitamin D deficiency could be involved in such pathologies as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type 1 diabetes, multiple sclerosis, cancer, cardiovascular and infectious diseases.
- Local modulation of the immune microenvironment requires high vitamin D doses. New pharmacologic forms are needed to avoid general toxicity, especially hypercalcemia.
- Randomized controlled trials will help to define new therapeutic modalities.

Summary points

- Vitamin D, beyond its role in bone metabolism, could be involved in many inflammatory diseases.
- Vitamin D receptor and the enzymes required for vitamin D metabolism are widely expressed in the immune system, and seem to induce an overall anti-inflammatory effect.
- Vitamin D deficiency is a frequent condition, with an increased prevalence in inflammatory diseases as shown in observational studies.
- Vitamin D supplementation has therapeutic effects in dysimmune disease models.
- The supplementation modalities remain unclear.

20. Inflammation and vitamin D

X. Guillot[1], C. Prati[1], N. Saidenberg-Kermanac'h[2], L. Semerano[2], G. Falgarone[2], M.C. Boissier[2] and D. Wendling[1]
[1]University Hospital Jean Minjoz, Rheumatology Department, Boulevard Fleming, 25030 Besançon. France; [2]University Hospital Avicenne, AP-HP, Rheumatology Department, 125 rue de Stalingrad, 93009 Bobigny cedex, France; xguillot@chu-besancon.fr

Abstract

Calcitriol, or 1,25-dihydroxyvitamin D3 is a well-known endocrine regulator of calcium homeostasis. More recently, local calcitriol production by immune cells was shown to exert autocrine or paracrine immunomodulating effects. Immune cells that produce calcitriol also express the vitamin D receptor and the enzymes required to metabolize vitamin D3 (1α-, 25- and 24-hydroxylases). These immunomodulating effects, shown in animal models and cell culture experiments, are both direct and indirect and involve T cells, B cells, and antigen presenting cells (dendritic cells and macrophages), affecting both innate and adaptative immunity. The overall effect is a switch from a Th1/Th17 to a Th2/Treg profile. These immunomodulating properties could explain the epidemiological associations, suggested by observational studies, between vitamin D serum levels and many autoimmune and inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel diseases, type 1 diabetes, but also infections, cancer, transplant rejection and cardiovascular diseases. Furthermore, vitamin D supplementation showed therapeutic effects in animal models for these diseases. Thus, vitamin D is a key focus for public health efforts and may hold promise for the treatment of dysimmune diseases.

Keywords: 1,25-dihydroxyvitamin D3, vitamin D receptor, 1α-hydroxylase, 25-hydroxylase, dendritic cell, macrophage, T cell, B cell

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_20, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
CD40L	CD40-ligand
COX-2	Cyclo-oxygenase 2
CRP	C-reactive protein
DCs	Dendritic cells
EAE	Experimental autoimmune encephalomyelitis
IBD	Inflammatory bowel disease
IFN-γ	Interferon gamma
IL-6	Interleukin-6
KO	Knockout
LT	T lymphocyte
mDC	Myeloid dendritic cells
MHC2	Class 2 major histocompatibility complex
MS	Multiple sclerosis
NFκB	Nuclear factor kappa B
NKT	Natural killer T cells
NOD	Nonobese diabetic
PGE_2	Prostaglandin E_2
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
Th	T helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Tr1	Type 1 regulatory T cells
Treg	Regulatory T cells
VDR	Vitamin D receptor

20.1 Introduction

Vitamin D is a secosteroid hormone mainly produced in the skin following exposure to ultraviolet B. It can also be provided by the diet or by supplements. The crucial role for vitamin D in calcium-phosphate homeostasis and bone metabolism is well established, with optimal serum levels considered to be at least of 30 ng/ml (75 nmol/l) in order to limit the release of parathyroid hormone and promote the intestinal absorption of calcium (Holick, 2007).

In order to become biologically active, cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) must be hydroxylated at position 25- in the liver and then at position 1- in the proximal tubule of the kidney, resulting in the production of the active form calcitriol ($1,25(OH)_2D$). This liposoluble hormone binds to VDR, which is widely expressed in targets relevant to calcium

homeostasis such as small bowel, osteoblasts. Vitamin D interacts with its target cells as a steroid hormone, by binding to its nuclear receptor VDR. VDR then undergoes heterodimerization, usually with the retinoid X receptor, and binds to specific DNA sequences (VDR elements) located in promoter regions, thereby controlling the transcription of target genes involved in calcium metabolism (e.g. binding proteins) and the immune response (Figure 20.1).

Recent observational studies suggest extra osseous effects of vitamin D. Indeed, vitamin D deficiency was associated with the risk and/or severity of many diseases including cancer, cardiovascular disease, sarcopenia, osteoarthritis, infections and transplant rejection, and notably autoimmune diseases such as diabetes, MS, and more recently IBD, and connective tissue diseases (mainly RA and SLE). These extra osseous effects of vitamin D, suggested by observational studies, are linked with vitamin D's immunomodulating properties, whose mechanisms have been shown in animal studies and cell culture experiments. Vitamin D influences the regulation of immune cells' proliferation, differenciation and function both in direct and indirect ways (Guillot *et al.*, 2010; Meyer *et al.*, 2011).

20.2 Immunomodulating effects of vitamin D

20.2.1 Enzyme metabolism

In addition to regulating calcium homeostasis, vitamin D has many other metabolic effects which were discovered more recently, notably via its immunomodulating properties first described about 20 years ago (Arnson *et al.*, 2007; Lemire *et al.*, 1984; Maruotti and Cantatore, 2010; Mora *et al.*, 2008; Rigby *et al.*, 1984; Sigmundsdottir *et al.*, 2007) (Table 20.1). Vitamin D can accumulate in the microenvironment of lymphoid organs, where it exerts specific autocrine and/or paracrine effects without unwanted systemic effects (such as hypercalcemia and increased bone resorption). Activated T (and probably B) cells can only hydroxylate 25(OH)D3 to 1,25$(OH)_2$D3, while macrophages and some DCs have both of the enzymes needed to convert vitamin D to

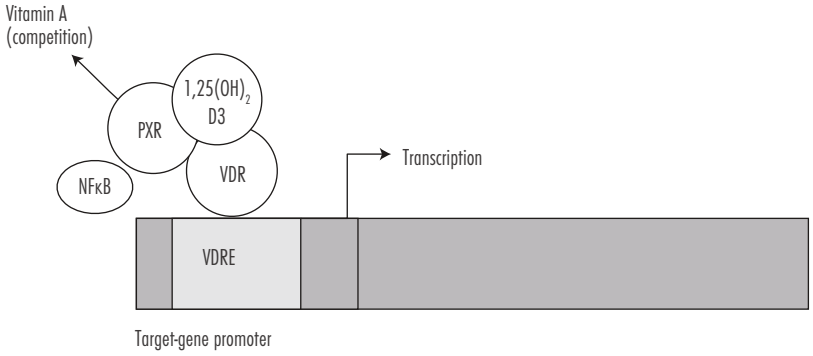

Figure 20.1. Mechanism of action of vitamin D within the nucleus of target cells.

Table 20.1. Effects of $1,25(OH)_2D3$ on immune cells.

Cell type	Effect
Myeloid dendritic cell	↓ proliferation, ↓ differenciation, ↓ survival, ↓ maturation
	↓ CD40, CD80, CD86,CMH-2: ↓ T cell stimulation
	↓ IL-12: Th1 response inhibition (indirect)
	↑ IL-10, ↑ Fox-P3: Treg induction
	↑ transforming growth factor-β
	↓ IL-23 (p40), ↓ Th17 induction
	↓ NFkB (p65) phosphorylation
Macrophage	↓ IL-6, ↓ IL-23: ↓ Th17 response
	↓ TNFa, ↓ IL-1
	↓ CMH2: ↓ antigen presentation
	↑ cathelicidin, phagocytosis,chemotactism: ↑ anti-infectious response
	↓ TLR9-4-2
	↓ COX-2, ↓ i-NOS, ↑ PGE2
T cell	↑ IL-5 +/-IL-4 transcription in T cells: ↑ Th2 response
	↑ NKT response
	↓ Th1 proliferation (direct), ↓ IL-2 and IFNγ (mRNA and proteins): ↓ Th1 response
	↑ IL-10-producing Tr1 cells
	↓ Th17 differentiation, ↓ IL-17 production
	homing
	↓ CD8+ T cells – cytotoxicity
	↓ Fas-ligand
B cell	↓ proliferation
	↓ plasma cell différenciation
	↓ memory B cells
	↓ immunoglobulin production (IgE)

$1,25(OH)_2D3$ (Fritsche *et al.*, 2003; Sigmundsdottir *et al.*, 2007) allowing $1,25(OH)_2D3$ to be produced locally and to exert paracrine effects. In contrast to renal cells, antigen-presenting cells are not subjected to negative regulation of 1-alpha-hydroxylase by parathyroid hormone nor by $1,25(OH)_2D3$. In antigen-presenting cells, 1-alpha-hydroxylase is induced by many factors, including IFNγ, and undergoes downregulation as the DC matures (Hewison *et al.*, 2003).

20.2.2 Role and expression of the vitamin D receptor

When activated by $1,25(OH)_2D3$, the VDR regulates gene expression in numerous tissues targeted by vitamin D. The VDR is expressed by many types of immune cells including circulating monocytes, macrophages, DCs, and activated T cells (Adams *et al.*, 1983; Proveddini *et al.*, 1983).

In VDR-KO mice, $1,25(OH)_2D3$ can no longer induce the differenciation of haematopoietic bone marrow progenitors to monocytes/macrophages, and plays a key-role in spleen cell Th1 response (O'Kelly *et al.*, 2002). However, VDR-KO mice have normal immune-cell populations and reject allogeneic and xenogeneic grafts as frequently as wild-type mice (Mathieu *et al.*, 2001). VDR-KO mice with induced asthma exhibit no airway inflammation, hypereosinophilia, nor bronchial hyperactivity despite high levels of immunoglobulin E and Th2 cytokines, suggesting a possible key-role of vitamin D in allergic response (Wittke *et al.*, 2004).

20.2.3 Effects on macrophages

Macrophages and DCs are regulated by $1,25(OH)_2D3$, which thereby plays an important role in innate immune responses. $1,25(OH)_2D3$ promotes monocyte-to-macrophage differenciation, stimulates macrophages to produce the immunosuppressant cytokine PGE_2 and inhibits granulocyte-macrophage colony stimulating factor expression (Towers *et al.*, 1999). It also down-regulates macrophage production of pro-inflammatory cytokines and chemokines (Zhang *et al.*, 2012), and enzymatic activities (COX-2, inducible NO-synthase) (Helming *et al.*, 2005). Vitamin D deficiency impairs macrophage maturation and the production of macrophage-specific membrane antigens, lysosomal acid phosphatase, and hydrogen peroxyd required for antimicrobial activity (Abu-Amer *et al.*, 1994). The addition of $1,25(OH)_2D3$ increases the expression of these membrane markers, enzymes and free oxygen radicals and up-regulates chemotaxis and phagocytosis (Goldman, 1984; Koeffler *et al.*, 1984). Activated macrophages produce $1,25(OH)_2D3$ in response to IFNγ and activation of the TLRs. TLR2 stimulation has been shown to induce the expression of CY27B1 gene, coding for 1α-hydroxylase (Schauber *et al.*, 2007). In human monocyte cultures, the addition of 100 nM of $1,25(OH)_2D3$ inhibits the expression of the innate-immunity receptors TLR2, TLR4 and TLR9 and impairs the TLR9-dependant production of IL-6 (Dickie *et al.*, 2010). Other effects of $1,25(OH)_2D3$ may in contrast stimulate innate immunity: indeed, it downregulates cell membrane expression of MHC2 antigens and induces the production of cathelicidin, a peptide involved in antimicrobial response (Cantorna and Mahon, 2004; Gombaert *et al.*, 2005; Liu *et al.*, 2006; Wang *et al.*, 2004). Furthermore, $1,25(OH)_2D3$-induced downregulation of the membrane expression of MHC2 can also impair the antigen-presenting function of macrophages.

20.2.4 Effects on myeloid dendritic cells

$1,25(OH)_2D3$ inhibits mDC proliferation, maturation, differenciation and survival, monocyte-to-DC differenciation, therefore decreasing mDC stimulating effect on LT (Griffin *et al.*, 2001; Penna *et al.*, 2005; Piemonti *et al.*, 2000). The NFκB transduction pathways is involved via vitamin D-induced inhibition of p65 subunit phosphorylation. $1,25(OH)_2D3$ also inhibits IL-12 production by mDC, subsequently indirectly inhibiting the Th1 response – the direct interaction between $1,25(OH)_2D3$, VDR and NFκB affecting IL-12 gene transcription (D'Ambrosio *et al.*, 1998). In addition to IL-12 inhibition, $1,25(OH)_2D3$ also increases IL-10 production by mDC. The overall result is a decrease in the Th1 response and probably an induction of IL-10-producing Tr1 cells. $1,25(OH)_2D3$ also modulates LT-DC interactions by acting on co-stimulatory molecules.

It down-regulates CD40L-induced pro-inflammatory cytokine production (notably IL-1 and TNF-α) and membrane MHC2, CD40, CD80, CD86 expression, causing a decreased production of IL-12 and IFNγ and an increased production of IL-10 and transforming growth factor-β (Adorini *et al.*, 2005; Almerighi *et al.*, 2009; Gauzzi *et al.*, 2005; Hardin, 2005; Lyach *et al.*, 2005). CD-40, CD-80 and CD-86 down-regulation in antigen presenting cells (notably in mDCs) induces a decreased activation of LT (Van Etten *et al.*, 2005). 1,25$(OH)_2$D3 can also induce autoreactive T cell apoptosis via PD-L receptor up-regulation and can promote CD4+CD25+ Treg induction by immature mDCs (Boissier *et al.*, 2009). In a murine colitis model, 1,25$(OH)_2$D3 increased the expression of Fox-P3 transcription factor and IL-10, two critical factors for Treg induction (Daniel *et al.*, 2008). This tolerogenic DC and Treg induction decreased the risk of rejection in a murine organ transplantation model (Gregori *et al.*, 2001).

20.2.5 Effects on T cells

Vitamin D exerts direct effects on T and B cells and alters their response to activation, thereby playing a key role in adaptative immune response. Quiescent CD4+ T cells express VDR at a low rate, which shows a five-fold increase after cell activation (Mahon *et al.*, 2003). To date, 102 target genes for 1,25$(OH)_2$D3 have been identified in CD4+ T cells (Mahon *et al.*, 2003). The effect of 1,25$(OH)_2$D3 on T cells is both direct via inhibition of T-cell proliferation, notably IFNγ and IL-2 producing Th1 cells (Daniel *et al.*, 2008; Van Etten *et al.*, 2005) and indirect: 1,25$(OH)_2$D3 inhibits the Th1 response by inhibiting DCs' IL-12 production (Penna and Adorini, 2000). Furthermore, 1,25$(OH)_2$D3 affects T-cell differenciation, inhibiting the Th1 response (cell proliferation, IFNγ and IL-2 gene transcription and protein expression) and stimulating the Th2 response (IL-4, IL-5 and IL-10) both indirectly by decreasing the production of IFNγ (Boonstra *et al.*, 2001, Van Etten *et al.*, 2005) and directly by increasing the production of IL-5 and IL-10 via an up-regulation of the Th2-related transcription factor GATA-3. The role of 1,25$(OH)_2$D3 in IL-4 regulation is more controversial (Cantorna *et al.*, 1998a; Staeva-Vieira and Freeman, 2002). In association with corticosteroids, 1,25$(OH)_2$D3 induces IL-10 producing-Tr1 cells, which repress the immune response and could in part explain vitamin D's therapeutic effects observed in animal models of autoimmune diseases (Barrat *et al.*, 2002). CD4+ T cells from VDR-KO mice produce more IFNγ and less IL-2, IL-4 and IL-5 compared to those from wild-type mice (Froicu *et al.*, 2003). In addition, 1,25$(OH)_2$D3 inhibits Th17 response both directly by inhibiting CD4+ differenciation into a Th17 phenotype and IL-17 synthesis (Liu *et al.*, 2006), and indirectly by repressing IL-6 and IL-23 synthesis (Daniel *et al.*, 2008). This decreased IL-23 production is linked with a vitamin D-induced down-regulation of the Th-17 related transcription factor RORγt (Mus *et al.*, 2010). In cell culture experiments, 1,25$(OH)_2$D3 induces Treg in the presence of IL-2, by up-regulating the transcription of Fox-P3 and CTLA4 genes (Jeffery *et al.*, 2009; Penna *et al.*, 2005). T cell homing is also affected by 1,25$(OH)_2$D3, which can act synergically with IL-12 to induce skin-homing marker CCR10 expression on T cell membrane surface, while keratinocytes express its ligand CCL27, which could promote T cell circulation and/or retention within epidermal tissues (Sigmundsdottir *et al.*, 2007). Conversely, 1,25$(OH)_2$D3 can also downregulate the expression of chemokines (CCL2, CXCL10) involved in pancreatic Th1 cell homing and infiltration in NOD mice, a model of type 1 diabetes (Giarrantana *et al.*, 2004) or in monocyte infiltration in EAE,

a murine model of MS (Pedersen *et al.*, 2007). In addition, $1,25(OH)_2D3$ inhibits CD8+ T cell-mediated cytotoxic response (Meehan *et al.*, 1992) and increases NKT cell function. In summary, $1,25(OH)_2D3$ inhibits the Th1 and Th17 proinflammatory responses and promotes the Th2, Treg and Tr1 immunomodulating responses, resulting in an overall downregulation of the effector T-cell immune response (Figure 20.2).

20.2.6 Effects on B cells

As concerns B cells, $1,25(OH)_2D3$ inhibits their proliferation, their differenciation into memory B cells, plasma cells, and immunoglobulin production, especially in SLE patients (Chen *et al.*, 2007; Lemire *et al.*, 1984; Linker-Israeli *et al.*, 2001).

20.3 Vitamin D and inflammatory diseases

Many studies have shown significantly lower vitamin D serum levels in patients with autoimmune diseases compared to healthy controls. Furthermore, vitamin D3 supplementation has been reported to prevent the onset or decrease severity of autoimmune diseases (Table 20.2), and relationships have been suggested between vitamin D deficiency and many autoimmune diseases (Holick, 2007).

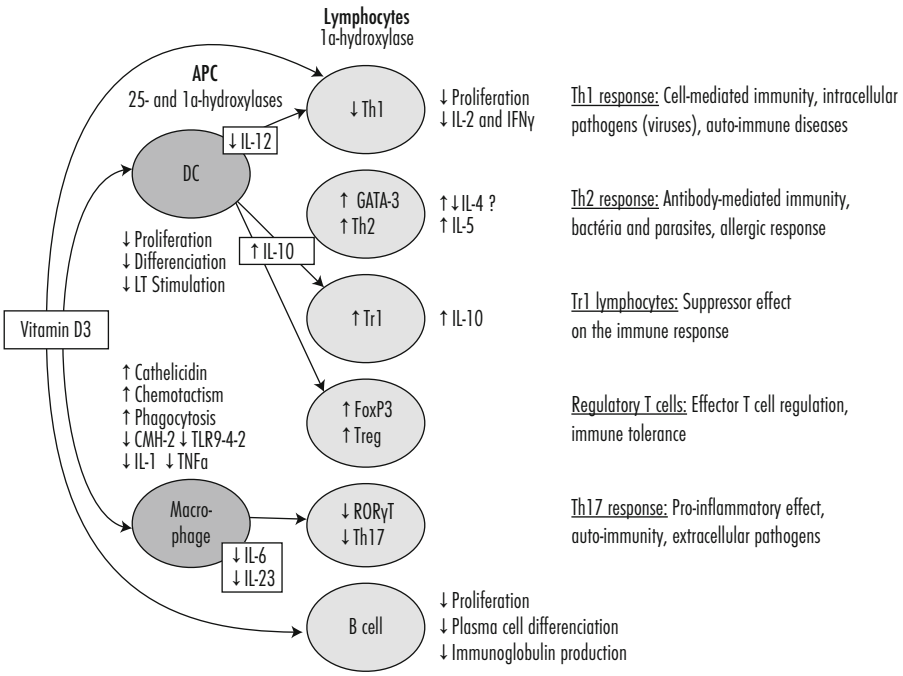

Figure 20.2. Vitamin D effects on immune cells.

Table 20.2. Vitamin D roles in auto-immune diseases.

	Vitamin D deficiency	Therapeutic effect (supplementation)	Animal models	Supplementation effect in these models
RA	More frequent Disease prevalence increases with latitude Correlates with disease activity (DAS 28 and CRP)	Pain CRP	Collagen-induced arthritis (mice)	Prevention Decreases arthritides
SLE	More frequent Correlates with disease activity (SLEDAI) Frequent in antiphospholipid syndrome (correlates with thrombotic event frequency)		MRL/lpr mice	Increases survival Decreases proteinuria
Type 1 diabetes	More frequent	Prevention	NOD mice	Blocks progression Regulatory t cell induction ↓ IL-12
MS	Disease prevalence increases with latitude Correlates with the risk of developing MS	Prevention ↓ Flare frequency	EAE (mice)	Preventive and therapeutic IL-10-mediated effect
IBD	More frequent Disease prevalence increases with latitude Relapse frequency	↓ Relapse frequency	IL-10 KO mice	Prevention Increases weight and survival Increased efficacy in association with calcium supplementation

20.3.1 Rheumatoid arthritis

Vitamin D deficiency is more prevalent in RA patients compared to the general population, but it is difficult to determine if this deficiency is a cause or a consequence of the disease (Aguado *et al.*, 2000; Als *et al.*, 1987; Ralston *et al.*, 1988). In the Iowa Women's Health Study, a higher vitamin D intake, assessed using a dietetic questionnaire, correlated with a lower RA frequency (Merlino *et al.*, 2004). However, a serum-bank case-control study from the Netherlands found no correlation between serum 25(OH)D3 levels and the onset of RA (Nielen *et al.*, 2006). In an evaluation of the Nurse's Health Study cohort including 190 SLE patients and 722 RA patients, there was no correlation between vitamin D intake estimated from semi-quantitative food frequency questionnaires and the risk of SLE or RA (Costenbader *et al.*, 2008). In RA patients, serum

25(OH)D3 levels correlated negatively with disease activity (Cutolo *et al.*, 2007; Patel *et al.*, 2007). In interventional trials, supplementation with 1 µg $1,25(OH)_2D3$ had no significant effect (Hein *et al.*, 2000), while higher doses were associated with reduced pain and significant CRP decrease (Andjelkovic *et al.*, 1999). In collagen-induced arthritis, a murine model of RA, supplementation with $1,25(OH)_2D3$ prevents arthritis development and blocks its progression (Boissier *et al.*, 1992; Cantorna *et al.*, 1998b). In cultured peripheral blood mononuclear cells from early RA patients, the supplementation with $1,25(OH)_2D3$ at the physiological concentration of 100 nM/l decreased IL-17, IFNγ and increased IL-4 production (Colin *et al.*, 2010). Vitamin D also decreased Th17-driven synovial fibroblast activation in early RA patients (Van Hamburg *et al.*, 2010), and acted synergically with TNF blockers to inhibit synovial inflammation (van Hamburg *et al.*, 2012). In human chondrocyte culture experiments, vitamin D metabolites act via the VDR to regulate the transcription of numerous genes involved in chondrocyte metabolism, resulting for instance in a modulation of proteoglycan and collagen synthesis, and the expression of specific matrix metalloproteinases (Dean *et al.*, 1997). In TNF transgenic mice, a murine model of arthritis, a KO for VDR gene aggravated clinical arthritides and synovial inflammation, with increased joint erosions, cartilage damages and an increased macrophage joint infiltration. VDR-KO macrophages showed a TNF-hyper responsive phenotype with prolonged MAP-kinase activation and cytokine secretion. VDR-KO monocytes also had an increased potential to differentiate into bone reabsorbing osteoclasts *in vitro* (Zwerina *et al.*, 2011). This suggests a key-role of vitamin D deficiency in RA activity and severity and potential therapeutic implications (Buckland, 2012).

20.3.2 Inflammatory bowel disease

The prevalence of IBD is higher in areas less exposed to sunlight, such as the northern parts of Europe and America (Cantorna, 2006; Podolsky, 1991; Sonnenberg and Wasserman, 1991). Patients with IBD have low 25(OH)D3 serum levels compared to controls (Jahnsen *et al.*, 2002). This result could be related to a combination of confounding factors including decreased intestinal absorption, lower dietary intakes, and limited sunlight exposure. Patients with newly diagnosed IBD also have lower serum 25(OH)D3 levels compared to controls (Lamb *et al.*, 2002). In a recent double-blind randomized controlled trial including 108 patients with Crohn's disease, daily oral supplementation with 1,200 UI of 25(OH)D3 increased the mean serum 25(OH)D3 levels from 69 to 96 nmol/l and reduced the relapse rates over the 1-year follow-up (13% vs. 29%; P=0,06) compared to the placebo (Jorgensen *et al.*, 2010). In IL-10 KO mice, vitamin D deficiency was associated with accelerated bowel inflammation, while vitamin D3 supplementation had therapeutic effects in this murine model of IBD (Cantorna, 2000; Cantorna *et al.*, 2000; Froicu *et al.*, 2003). In a murine model of Th1-mediated experimental colitis, a low calcemic vitamin D analog exerted both preventive and therapeutic effects (Daniel *et al.*, 2006).

20.3.3 Systemic lupus erythematosus

In the US, African Americans have a three-fold higher incidence of SLE with earlier disease onset and higher mortality and morbidity rates compared to Caucasians (Alarcon *et al.*, 1999). A genetic factor is unlikely involved, as SLE is rare in Western Africa. This result is more probably related to

a reduced sunlight exposure and a decreased cutaneous penetration of ultraviolet B radiation due to high melanine levels. Vitamin D deficiency is common in SLE patients, whatever the disease duration, but it might be a consequence of the disease, related to an increased photoprotection (Bultink *et al.*, 2005; Kamen *et al.*, 2006; Muller *et al.*, 1995). Conversely, a cross-sectional study found no significant difference in serum 25(OH)D3 between 25 women with SLE and 25 women with fibromyalgia (Huisman *et al.*, 2001). In one study, serum 25(OH)D3 levels were lower in patients with undifferentiated connective tissue diseases compared to controls and vitamin D deficiency seemed to be involved in the subsequent development of differentiated connective tissue diseases in these patients such as RA, Sjögren's syndrome, SLE, overlap syndromes, systemic vasculitides, and antiphospholipid syndrome. In addition, skin and pleural involvement correlated negatively with serum 25(OH)D3 levels in this study (Zold *et al.*, 2008). In murine models of SLE, vitamin D supplementation had therapeutic effects, decreasing proteinuria and increasing life span (Lemire *et al.*, 1992; Vaisberg *et al.*, 2000).

20.3.4 Diabetes and multiple sclerosis

In NOD mice, a model of diabetes, administration of a $1,25(OH)_2D3$ analog diminished the expression of IL-12 and IL-12 and IFNγ, prevented DC maturation and pancreatic islet infiltration by Th1 cells, and halted disease progression (Mathieu *et al.*, 1992). Treg cells were probably involved in this effect, as they were found in increased numbers in the pancreas-draining lymph-nodes (Gregori *et al.*, 2002).

Vitamin D supplementation also showed a therapeutic effect in an animal model of autoimmune thyroiditis (Fournier *et al.*, 1990). Vitamin D supplementation showed preventive and therapeutic effects in EAE, a murine model of MS (Lemire and Archer, 1991; Van Etten *et al.*, 2003). These effects were mediated by IL-10 (Spach *et al.*, 2006).

20.3.5 Transplantation, cardiovascular and infectious diseases

Vitamin D could be used as an adjuvant therapy in association with immunomodulating drugs in organ transplantation. In a murine model of heart allograft, $1,25(OH)_2D3$ and one of its analogs prolonged allograft survival (Amuchastegui *et al.*, 2005). Furthermore, even if $1,25(OH)_2D3$ contributes to prevent graft rejection, it does not seem to interfere significantly with the protective anti-infectious immune response (Cantorna *et al.*, 1998c). Vitamin D deficiency seems to affect blood pressure and myocardial function in a possibly TNF-α-dependant fashion (Zittermann *et al.*, 2003). $1,25(OH)_2D3$ is also able to induce antimicrobial peptide gene expression (Wang *et al.*, 2004).

20.4 Therapeutic implications

The optimal serum 25(OH)D3 concentration for bone health should be at least 30 ng/ml (75 nmol/l), with the ideal range being estimated at 30-60 ng/ml. Higher serum levels could

be required for vitamin D to exert its beneficial extra osseous effects (Meyer *et al.*, 2011). Vitamin D poisoning occurs at levels greater than 150 ng/ml (Holick, 2007). Only fragmentary information is available on the serum 25(OH)D3 concentration required for vitamin D to exert immunomodulating effects. In one observational study, the risk of multiple sclerosis decreased by 41% for each 20 ng/ml increase in serum 25(OH)D3, starting at a threshold level of about 24 ng/ml (60 nmol/l) (Munger *et al.*, 2004). When serum 25(OH)D levels fall below 20 ng/ml (50 nmol/l), human monocytes and macrophages are unable to initiate some of the innate immune responses *in vitro*, which could explain the increased risk of tuberculosis in populations with prevalent vitamin D deficiency such as African-Americans (Liu *et al.*, 2006). No serum 25(OH)D threshold has been documented for RA. However, in one observational study, each 10 ng/ml increase in serum 25(OH)D was associated with a 0,3-point decrease in mean DAS28 and a 25% decrease in serum CRP (Patel *et al.*, 2007). The pleiotropic roles of vitamin D in the immune response suggest it could be used as a therapeutic agent in many autoimmune diseases. The incidence and severity of such pathologies as RA, type 1 diabetes, IBD and MS could be reduced by increasing vitamin D intake (Cantorna and Mahon, 2004). In active RA patients, supplementation with α-calcidiol (2 µg/day) improved inflammation parameters (Andjelkovic *et al.*, 1999). Obtaining immunomodulating effects *in vitro* requires local concentrations of $1,25(OH)_2D3$ of about 10^{-10} M in the immune microenvironment. The obtention of these concentrations requires supraphysiological doses of vitamin D3, associated with a non-acceptable risk of hypercalcemia. Therefore, vitamin D analogs inducing no hypercalcemia have been developed (Baeke *et al.*, 2011; Brown and Slatopolsky, 2008; Plum and DeLuca, 2010). Vitamin D can also be used synergistically with immunomodulating drugs such as ciclosporine or with bisphosphonates (Daniel *et al.*, 2006; Van Etten *et al.*, 2005). *In vivo* studies of murine models suggest that not only vitamin D but also a sufficient calcium intake contribute to modulate the immune response in the gastrointestinal tract and in the central nervous system. Concomitant calcium supplementation enhances the ability of vitamin D to prevent IBD and MS (Cantorna *et al.*, 1999,2000).

20.5 Conclusion

Vitamin D's immunomodulating properties may hold therapeutic promise in a wide range of inflammatory diseases, including autoimmune diseases, infections, malignancies and cardiovascular diseases. These immunomodulating effects could be related to anti-Th1 and anti-Th17, pro-Th2 and Treg/Tr1-inducing effects (Boissier *et al.*, 2008). Treatment modalities (doses, target levels, pharmacological forms, association with calcium) remain to be established in randomized controlled trials.

References

Abu-Amer, Y. and Bar-Shavit, Z., 1994. Regulation of TNF-alpha release from bone marrow-derived macrophages by vitamin D. J Cell Biochem 55, 435-444.

Adams, J.S., Sharma, O.P., Gacad, M.A. and Singer, F.R., 1983. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 72, 1856-1860.

Adorini, L., 2005. Intervention in autoimmunity: the potential of vitamin D receptor agonists. Cell Immunol 233, 115-124.

Aguado, P., del Campo, M.T., Garcés, M.V., González-Casaús, M.L., Bernad, M., Gijón-Baños J., Martín Mola, E., Torrijos, A. and Martínez, M.E., 2000. Low vitamin D levels in outpatient postmenopausal women from a rheumatology clinic in Madrid, Spain: their relationship with bone mineral density. Osteoporos Int 11, 739-744.

Alarcon, G.S., Rodríguez, J.L., Benavides Jr., G., Brooks, K., Kurusz, H. and Reveille J.D., 1999. Systemic lupus erythematosus in three ethnic groups: III. A comparison of characteristics early in the natural history of the LUMINA cohort. Lupus in Minority populations: Nature vs, Nurture. Lupus 8, 197-209.

Almerighi, C., Sinistro, A., Cavazza, A.,. Ciaprini, C., Rocchi, G. and Bergamini, A., 2009. 1a,25-dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in human monocytes. Cytokine 45, 190-197.

Als, OS., Riis, B. and Christiansen, C., 1987. Serum concentration of vitamin D metabolites in rheumatoid arthritis. Clin Rheumatol 6, 238-243.

Amuchastegui, S., Daniel, K.C. and Adorini, L., 2005. Inhibition of acute and chronic allograft rejection in mouse models by BXL-628, a nonhypercalcemic vitamin D receptor agonist. Transplantation 80, 81-87.

Andjelkovic, Z., Vojinovic, J., Pejnovic, N., Popovic, M., Dujic, A., Mitrovic, D., Pavlica, L. and Stefanovic, D., 1999. Disease modifying and immunoregulatory effects of high dose 1 alpha (OH) D3 in rheumatoid arthritis patients. Clin Exp Rheumatol 17, 453-456.

Arnson, Y., Amital, H. and Shoenfeld, Y., 2007. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. Ann Rheum Dis, 66, 1137-1142.

Baeke, F., Korf, H., Overbergh, L., Verstuyf, A., Thorrez, L., Van Lommel, L., Waer, M., Schuit, F., Gysemans, C. and Mathieu, C., 2011. The Vitamin D Analog, TX527, Promotes a Human CD4+CD25highCD127low Regulatory T Cell Profile and Induces a Migratory Signature Specific for Homing to Sites of Inflammation. J Immunol 186, 132-142.

Barrat, F.J., Cua, D.J., Boonstra, A., Richards, D.F., Crain, C., Savelkoul, H.F., De Waal-Malefyt, R., Coffman, R.L., Hawrylowicz, C.M. and O'Garra, A., 2002. *In vitro* generation of interleukin 10-producing regulatory CD4+ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1) and Th2-inducing cytokines. J. Exp. Med 195, 603-616.

Boissier, M.C., Assier, E., Falgarone, G. and Bessis, N., 2008. Shifting the imbalance from Th1/Th2 to Th17/Treg: the changing rheumatoid arthritis paradigm. Joint Bone Spine 75, 373-375.

Boissier, M.C., Assier, E., Biton, J., Denys, A., Falgarone, G. and Bessis N., 2009. Regulatory T cells (Treg) in rheumatoid arthritis. Joint Bone Spine 76, 10-14.

Boissier, M.C., Chiocchia, G. and Fournier, C., 1992. Combination of cyclosporine A and calcitriol in the treatment of adjuvant arthritis. J Rheumatol 19, 754-757.

Boonstra, A., Barrat, F.J., Crain, C., Heath, V.L., Savelkoul, H.F. and O'Garra, A., 2001. 1alpha,25 Dihydroxyvitamin D3 has a direct effect on naive CD4+ T cells to enhance the development of Th2 cells. J Immunol 167, 4974-4980.

Brown, A.J. and Slatopolsky, E., 2008. Vitamin D analogs: therapeutic applications and mechanisms for selectivity. Mol Aspects Med 29, 433-452.

Buckland, J., 2012. Rheumatoid arthritis: control of T(H)17 cell activity in RA by a combination of vitamin D receptor signaling and TNF-blockade. Nat Rev Rheumatol 24, 124.

Bultink, I.E., Lems, W.F., Kostense, P.J., Dijkmans, B.A. and Voskuyl, A.E., 2005. Prevalence of and risk factors for low bone mineral density and vertebral fractures in patients with systemic lupus erythematosus. Arthritis Rheum 52, 2044-2050.

Cantorna, M.T., 2000. Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? Proc Soc Exp Biol Med 223, 230-233.

Cantorna, M.T., 2006. Vitamin D and its role in immunology: multiple sclerosis, and inflammatory bowel disease. Prog Biophys Mol Biol 92, 60-64.

Cantorna, M.T. and Mahon, B.D., 2004. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp Biol Med 229, 1136-1142.

Cantorna, M.T., Hayes, C.E. and DeLuca, H.F., 1998b. 1,25 Dihydroxycholecalciférol inhibits the progression of arthritis in murine models of human arthritis. J Nutr 128, 68-72.

Cantorna, M.T., Hullett D.A, Redaelli C, Brandt C.R, Humpal-Winter J, Sollinger H.W, Deluca H.F., 1998c. 1,25-dihydroxyvitamin D3 prolongs graft survival without compromising host resistance to infection or bone mineral density. Transplantation 66, 828-831.

Cantorna, M.T., Humpal-Winter, J. and DeLuca, H.F, 1999. Dietetary calcium is a major factor in 1,25-Dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. J Nutr 129, 1966-1971.

Cantorna, M.T., Munsick, C., Bemiss, C. and Mahon, B.D., 2000. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr 130, 2648-2652.

Cantorna, M.T., Woodward, W.D., Hayes, C.E., DeLuca, H.F., 1998a. 1,25-dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-beta1 and IL-4. J Immunol 160, 5314-5319.

Chen, S., Sims, G.P., Chen, X.X., Gu, Y.Y., Chen, S. and Lipsky, P.E., 2007. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differenciation. J Immunol 179, 1634-1647.

Colin, E.M., Asmawidjaja, P.S., Van Hamburg, J.P., Mus, A.M., Van Driel, M., Hazes, J.M., Van Leeuwen, J.P. and Lubberts, E., 2010. 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. Arthritis Rheum 62, 132-142.

Costenbader, K.H., Feskanich, D., Holmes, M., Karlson, E.W. and Benito-Garcia, E., 2008. Vitamin D intake and risks of lupus erythematosus and rheumatoid arthritis in women. Ann Rheum Dis 67, 530-535.

Cutolo, M., Otsa, K., Uprus, M., Paolino, S. and Seriolo, B., 2007. Vitamin D in rheumatoid arthritis. Autoimmun. Rev 7, 59-64.

D'Ambrosio, D., Cippitelli, M., Cocciolo, M.G., Mazzeo, D., Di Lucia, P., Lang, R., Sinigaglia, F. and Panina-Bordignon, P., 1998. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvment of NF-kappaB downregulation in transcriptional repression of the p40 gene. J Clin Invest 101, 252-262.

Daniel, C., Radeke, H.H., Sartory, N.A., Zahn, N., Zuegel, U., Steinmeyer, A. and Stein, J., 2006. The new low calcemic vitamin D analog 22-ene-25-oxa-vitamin D prominently ameliorates Th1-mediated colitis in mice. J Pharmacol Exp Ther 319, 622-631.

Daniel, C., Sartory, N.A., Zahn, N., Radeke, H.H. and Stein, J.M., 2008. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th 17 to a Th 2 and regulatory T cell profile. J Pharmacol Exp Ther 324, 23-33.

Dean, D.D., Schwartz, Z., Muniz, O.E., Arsenis, C.H., Boyan, B.D. and Howell, D.S., 1997. Interleukin-1α and β in growth plate cartilage are regulated by vitamin D metabolites. J Bone Miner Res 12, 1560-1569.

Dickie, L.J., Church, L.D., Coulthard, L.R., Mathews, R.J., Emery, P. and McDermott, M.F., 2010. Vitamin D3 down-regulates intracellular Toll-like receptor 9-induced IL-6 production in human monocytes. Rheumatology (Oxford) 30, 1-6.

Fournier, C., Gepner, P., Sadouk, M. and Charreire, J., 1990. *In vivo* beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D3 on the induction of experimental autoimmune thyroiditis. Clin Immunol Immunopathol 54, 53-63.

Fritsche, J., Mondal, K., Ehrnsperger, A. andreesen, R. and Kreutz, M., 2003. Regulation of 25-hydroxyvitamin D3-1α hydroxylase and production of 1α,25-dihydroxyvitamin D3 by human dendritic cells. Blood, 102, 3314-3316.

Froicu, M., Weaver, V., Wynn, T.A., McDowell, M.A., Welsh, J.E. and Cantorna, M.T., 2003. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Mol Endocrinol 17, 2386-2392.

Gauzzi, M.C., Purificato, C., Donato, K., Jin, Y., Wang, L., Daniel, K.C., Maghazachi, A.A., Belardelli, F., Adorini, L. and Gessani, S., 2005. Suppressive effect of 1 alpha,25-dihydroxyvitamin D3 on typeI IFN-mediated monocyte differenciation into dendritic cells: impairment of functional activities and chemotaxis. J Immunol 174, 270-276.

Giarrantana, N., Penna, G., Amuchastegui, S., Mariani, R., Daniel, K.C. and Adorini, L., 2004. Vitamin D analog down-regulates proinflammatory chemokine production by pancreatic islets inhibiting T cell recruitment and type 1 diabetes development. J Immunol 173, 2280-2287.

Goldman, R., 1984. Induction of a high phagocytic capability in P388D1, a macrophage-like tumor cell line, by 1 alpha-dihydroxyvitamin D3. Cancer Res 44, 11-19.

Gombaert, A.F., Borregaard, N. and Koeffler, H.P., 2005. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly upregulated in myeloid cells by 1,25-dihydroxyvitamin D3. Faseb J 19, 1067-1077.

Gregori, S., Casorati, M., Amuchastegui, S., Smiroldo, S., Davalli, A.M. and Adorini, L., 2001. Regulatory T cells induced by 1 alpha,25 dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. J Immunol 167, 1945-1953.

Gregori, S., Giarratana, N., Smiroldo, S., Uskokovic, M. and Adorini, L., 2002. 1 alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes 51, 1367-1374.

Griffin, M.D., Lutz, W., Phan, V.A., Bachman, L.A., McKean, D.J. and Kumar, R., 2001. Dendritic cell modulation by 1 alpha, 25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependant pathway that promotes a persistent state of immaturity *in vitro* and *in vivo*. Proc Natl Acad Sci USA 98, 6800-6805.

Guillot, X., Semerano, L., Saidenberg-Kermanac'h, N., Falgarone, G. and Boissier, M.C., 2010. Vitamin D and inflammation. Joint Bone Spine 77, 552-557.

Hardin, JA., 2005. Dendritic cells: potential triggers of autoimmunity and targets for therapy. Ann Rheum Dis 64, 86-90.

Hein, G. and Oelzner, P., 2000. Vitamin D metabolites in rheumatoid arthritis: findings-hypotheses-consequences. Z Rheumatol 59, 28-32.

Helming, L., Böse, J., Ehrchen, J., Schiebe, S., Frahm, T., Geffers, R., Probst-Kepper, M., Balling, R. and Lengeling, A., 2005. 1alpha-25-Dihydroxyvitamin D3 is a potent suppressor of interferon gamma-mediated macrophage activation. Blood 106, 4351-4358.

Hewison, M., Freeman, L., Hughes, SV., Evans, K.N., Bland, R., Eliopoulos, A.G., Kilby, M.D., Moss, PA. and Chakraverty, R., 2003. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol 170, 5382-5390.

Holick, M.F., 2007. Vitamin D deficiency. N Engl J Med 357, 266-281.

Huisman, A.M., White, K.P., Algra, A., Harth, M., Vieth, R., Jacobs, J.W., Bijlsma, J.W. and Bell, D.A., 2001. Vitamin D levels in women with systemic lupus erythematosus and fibromyalgia. J Rheumatol 28, 2535-2539.

Jahnsen, J., Falch, J.A., Mowinckel, P. and Aadland, E., 2002. Vitamin D status, parathyroid hormon and bone mineral density in patients with inflammatory bowel disease. Scand J Gastroenterol 37, 192-199.

Jeffery, L.E., Burke, F., Mura, M., Zheng, Y., Qureshi, O.S., Hewison, M., Walker, L.S., Lammas, D.A., Raza, K. and Sansom, D.M., 2009. 1,25-dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol 183, 5458-5467.

Jorgensen, S.P., Agnholt, J., Glerup, H., Lyhne, S., Villadsen, G.E., Hvas, CL., Bartels, L.E., Kelsen, J., Christensen, L.A. and Dahlerup, J.F., 2010. Clinical trial: vitamin D3 treatment in Crohn's disease – a randomised double-blind placebo-controlled study. Aliment Pharmacol Ther 32, 377-383.

Kamen, D.L., Cooper, G.S., Bouali, H., Shaftman, S.R., Hollis, B.W. and Gilkeson, G.S., 2006. Vitamin D deficiency in systemic lupus erythematosus. Autoimmun Rev 5, 114-117.

Koeffler, H.P., Amatruda, T., Ikekawa, N., Kobayashi, Y. and DeLuca, H.F., 1984. Induction of macrophage differenciation of human normal and leukemic myeloid stem cells by 1,25-dihydroxyvitamin D3 and its fluorinated analogs. Cancer Res 44, 5624-5628.

Lamb, EJ., Wong, T., Smith, D.J., Simpson, D.E., Coakley, A.J., Moniz, C. and Muller, A.F., 2002. Metabolic bone disease is present at diagnosis in patients with inflammatory bowel disease. Aliment Pharmacol Ther 16, 1895-1902.

Lemire, J.M., Adams, J.S., Sakai, R. and Jordan, S.C., 1984. 1α,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J. Clin. Invest 74, 657-661.

Lemire, J.M. and Archer, D.C., 1991. 1,25-dihydroxyvitamin D3 prevents the *in vivo* induction of murine experimental autoimmune encephalomyelitis. J Clin Invest 87, 1103-1107.

Lemire, J.M., Ince, A. and Takashima, M., 1992. 1,25-Dihydroxyvitamin D3 attenuates the expression of experimental murine lupus of MRL/1 mice. Autoimmunity 12, 143-148.

Linker-Israeli, M., Elstner, E., Klinenberg, J.R., Wallace, D.J. and Koeffler, H.P., 2001. Vitamin D3 and ist synthetic analogs inhibit the spontaneous *in vitro* immunoglobulin production by SLE-derived PBMC. Clin Immunol 99, 82-93.

Liu, P.T., Stenger, S., Li, H., Wenzel, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., Kamen, D.L., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R.L., Eisenberg, D., Hewison, M., Hollis, B.W., Adams, J.S., Bloom, B.R. and Modlin, R.L., 2006. toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311, 1770-1773.

Lyach, L.A., Sanford, M., Chekol, S., Young, H.A. and Roberts, A.B., 2005. TGF-beta and vitamin D3 utilize distinct pathways to suppress IL-12 production and modulate rapid differenciation of human monocytes into CD83+ dendritic cells. J Immunol 174, 2061-2070.

Mahon, B.D., Wittke, A., Weaver, V. and Cantorna, M.T., 2003. The targets of vitamin D depend on the differenciation and activation status of CD4 positive T cells. J Cell Biochem 89, 922-932.

Maruotti, N. and Cantatore F.P., 2010. Vitamine D and the immune system. J Rheumatol 37, 1-5.

Mathieu, C., Laureys, J., Sobis, H., Vandeputte, M., Waer, M. and Bouillon, R., 1992. 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice. Diabetes 41, 1491-1495.

Mathieu, C., Van Etten, E., Gysemans, C., Decallonne, B., Kato, S., Laureys, J., Depovere, J., Valckx, D., Verstuyf, A. and Bouillon, R., 2001. *In vitro* and *in vivo* analysis of the immune system of vitamin D receptor knockout mice. J Bone Miner Res 16, 2057-2065.

Meehan, M.A., Kerman, R.H. and Lemire, J.M., 1992. 1,25-dihydroxyvitamin D3 enhances the generation of nonspecific suppressor cells while inhibiting the induction of cytotoxic cells in a human MLR. Cell Immunol 140, 400-409.

Meyer, O., Dawidowicz, K. and Wabo, S., 2011. L'Actualité rhumatologique 2011. Elsevier Masson Publishing, Paris, France, 243 pp.

Merlino, L.A., Curtis, J., Mikuls, T.R., Cerhan, J.R., Criswell, L.A. and Saag, K.G., 2004. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum 50, 72-77.

Mora, J.R., Iwata, M. and Von Andrian, U.H., 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat. Rev. Immunol. 8, 685-698.

Muller, K., Kriegbaum, N.J., Baslund, B., Sørensen, O.H., Thymann, M. and Bentzen, K., 1995. Vitamin D3 metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin D3 in patients with systemic lupus erythematosus. Clin Rheumatol 14, 397-400.

Munger, K.L., Zhang, S.M., O'Reilly, E., Hernán, M.A., Olek, M.J., Willett, W.C. and Ascherio, A., 2004. Vitamin D intake and incidence of multiple sclerosis. Neurology 62, 60-65.

Mus, A.M., Van Hamburg, J.P., Asmawidjaja, P., Hazes J.M.W., Van Leeuwen, H., Boon, L., Colin, E. and Lubberts, E., 2010. Vitamin D suppresses Th17 cytokines via down regulation of RORgammat and NFATC2 and by differencial regulation of GATA3. Arthritis Rheum 62 (suppl), S14, abstract 38.

Nielen, M.M., Van Schaardenburg, D., Lems, W.F., Van de Stadt, R.J., De Koning, M.H., Reesink, H.W., Habibuw, M.R., Van der Horst-Bruinsma, I.E., Twisk, J.W. and Dijkmans, B.A., 2006. Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino and al. Arthritis Rheum 54, 3719-3720.

O'Kelly, J., Hisatake, J., Hisatake, Y., Bishop, J., Norman, A. and Koeffler, H.P., 2002. Normal myelopoiesis but abnormal T lymphocyte responses in vitamin D receptor knockout mice. J Clin Invest 109, 1091-1099.

Patel, S., Farragher, T., Berry, J., Bunn, D., Silman, A. and Symmons, D., 2007. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum 56, 2143-2149.

Pedersen, L.B., Nashold, F.E., Spach, K.M. and Hayes, C.E., 2007. 1,25-dihydroxyvitamin D3 reverses experimental autoimmune encephalomyelitis by inhibiting chemokine synthesis and monocyte trafficking. J. Neurosci. Res. 85, 2480-2490.

Penna, G. and Adorini, L., 2000. 1(alpha),25-dihydroxyvitamin D3 inhibits differenciation, maturation, activation and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 164, 2405-2411.

Penna, G., Roncari, A., Amuchastegui, S., Daniel, K.C., Berti, E., Colonna, M. and Adorini, L., 2005. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for function of CD4+FoxP3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood 106, 3490-3497.

Piemonti, L., Monti, P., Sironi, M., Fraticelli, P., Leone, B.E., Dal Cin, E., Allavena, P. and Di Carlo, V., 2000. Vitamin D3 affects differenciation, maturation, and function of human monocyte-derived dendritic cells. J Immunol 164, 4443-4451.

Plum, L.A. and DeLuca, H.F., 2010. Vitamin D, disease and therapeutic opportunities. Nat Rev Drug Discov 9, 941-955.

Podolsky, DK., 1991. Inflammatory bowel disease (1). N Engl J Med 325, 928-937.

Proveddini, D.M., Tsoukas, C.D., Deftos, L.J. and Manolagas, S.C., 1983. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. Science 221, 1181-1183.

Ralston, S.H., Willocks, L., Pitkeathly, D.A., Morton, R. and Smith, G.D., 1988. High prevalence of unrecognized osteomalacia in hospital patients with rheumatoid arthritis. Br J Rheumatol 27, 202-205.

Rigby, W.F., Stacy, T. and Fanger, M.W., 1984. Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D3 (calcitriol). J. Clin. Invest 1984, 74, 1451-1455.

Schauber, J., Dorschner, R.A., Coda, A.B., Büchau, A.S., Liu, P.T., Kiken, D., Helfrich, Y.R., Kang, S., Elalieh, H.Z., Steinmeyer, A., Zügel, U., Bikle, D.D., Modlin, R.L. and Gallo, R.L., 2007. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependant mechanism. J Clin Invest 117, 803-811.

Sigmundsdottir, H., Pan, J., Debes, G.F., Alt, C., Habtezion, A., Soler, D. and Butcher, E.C., 2007. DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. Nat. Immunol. 8, 285-293.

Sonnenberg, A. and Wasserman, I.H., 1991. Epidemiology of inflammatory bowel disease among US military veterans. Gastroenterology 101, 122-130.

Spach, K.M., Nashold, F.E., Dittel, B.N. and Hayes, C.E., 2006. IL-10 signaling is essential for 1,25-dihydroxyvitamin D3-mediated inhibition of experimental autoimmune encephalomyelitis. J Immunol 177, 6030-6037.

Staeva-Vieira, T.P. and Freeman, L.P., 2002. 1,25-dihydroxyvitamin D3 inhibits IFN-gamma and IL-4 levels during *in vitro* polarization of primary murine CD4+ T cells. J Immunol 168, 1181-1189.

Towers, T.L., Staeva, T.P. and Freedman, L.P., 1999. A two-hit mechanism for vitamin D3-mediated transcriptional repression of the granulocyte-macrophage colony-stimulating factor gene: vitamin D receptor competes for DNA binding with NFAT1 and stabilizes c-jun. Mol Cell Biol 19, 4191-4199.

Vaisberg, M.W., Kaneno, R., Franco, M.F. and Mendes, N.F., 2000. Influence of cholecalciferol (vitamin D3) on the course of experimental systemic lupus erythematosus in F1 (NZB X W) mice. J clin Lab Anal 14, 91-96.

Van Etten, E., Branisteanu, D.D., Overbergh, L., Bouillon, R., Verstuyf, A. and Mathieu, C., 2003. Combination of a 1,25-dihydroxyvitamin D3 analog and a bisphosphonate prevents experimental autoimmune encephalomyelitis and preserves bone. Bone 32, 397-404.

Van Etten, E. and Mathieu, C., 2005. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol 97, 93-101.

Van Hamburg, J.P., Asmawidjaja, P., Mus, A.M., Hazes, M., Van Leeuwen, H., Colin, E. and Lubberts, E., 2010. Vitamin D suppresses the pathogenic behavior of primary Th17 cells from patients with early RA in synovial fibroblast activation. Arthritis Rheum 62 (suppl), S530 (abstract 1274).

Van Hamburg, J.P., Asmawidjaja, P.S., Davelaar, N., Mus, A.M., Cornelissen, F., Van Leeuwen, J.P., Hazes, J.M., Dolhain, R.J., Bakx, P.A., Colin, E.M. and Lubberts, E., 2012. TNF blockade requires 1,25 (OH)2 D3 to control human Th17-mediated synovial inflammation. Ann Rheum Dis 71, 606-612.

Wang, T.T., Nestel, F.P., Bourdeau, V., Nagai, Y., Wang, Q., Liao, J., Tavera-Mendoza, L., Lin, R., Hanrahan, J.W., Mader, S. and White, J.H., 2004. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173, 2909-2912.

Wittke, A., Weaver, V., Mahon, B.D., August, A. and Cantorna, M.T., 2004. Vitamin D-deficient mice fail to develop experimental allergic asthma. J Immunol 173, 3432-3436.

Zhang, Y., Leung, D.Y., Richers, B.N., Liu, Y., Remigio, L.K., Riches, D.W. and Goleva, E., 2012. Vitamin D Inhibits Monocyte/Macrophage Proinflammatory Cytokine Production by Targeting MAPK Phosphatase-1. J Immunol 188, 2127-2135.

Zittermann, A., Schleithoff, S.S., Tenderich, G., Berthold, H.K., Körfer, R. and Stehle, P., 2003. Low vitamin D status: A contributing factor in the pathogenesis of congestive heart failure?. J Am Coll Cardiol 41, 105-112.

Zold, E., Szodoray, P., Gaal, J., Kappelmayer, J., Csathy, L., Gyimesi, E., Zeher, M., Szegedi, G. and Bodolay, E., 2008. Vitamin D deficiency in undifferenciated connective tissue disease. Arthritis Res Ther10, R123.

Zwerina, K., Baum, W., Axmann, R., Heiland, G.R., Distler, J.H., Smolen, J., Hayer, S., Zwerina, J. and Schett, G., 2011. Vitamin D receptor regulates TNF-mediated arthritis. Ann Rheum Dis 70, 1122-1129.

Vitamin D and cancer

Key facts

- Pancreatic cancer is one of the most lethal cancers.
- Vitamin D has functions other than maintaining good bones.
- Vitamin D deficiency is a risk factor for pancreatic cancer and other cancers.
- Vitamin D analogs have potential to treat advanced pancreatic cancer.

Summary points

- Risk factors for pancreatic cancer are smoking, heavy alcohol consumption, obesity and diabetes mellitus, among others.
- The anti-tumor actions of 1a,25$(OH)_2$D and its analogs may include pro-apoptosis, anti-angiogenesis, anti-inflammatory in addition to anti-proliferation and pro-differentiation.
- The vitamin D analog, MART-10 is about 1000-fold more active than 1a,25$(OH)_2$D3 in inhibiting the proliferation of LNCaP and PC-3 prostate cancer cell lines and HepG2 liver cancer cell line *in vitro*.

21. Vitamin D in the prevention and treatment of pancreatic cancer

T.C. Chen[1] and K.C. Chiang[2]
[1]Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA; [2]Department of Surgery, Chang Gung Memorial Hospital and Chang Gung University, Keelung, Taiwan;
taichen@bu.edu

Abstract

Pancreatic cancer is one of the most lethal cancers with a 5-year survival rate of less than 5%. The more aggressive nature of this disease contributes to its usual late stage diagnosis that makes most patients unsuitable for receiving curative surgery. In addition, traditional chemotherapy and radiotherapy are not effective in this group of patients, leaving little therapeutic choices for them. 1α,25-dihydroxyvitamin D3 (1α,25$(OH)_2$D3), the biologically active form of vitamin D3, is now recognized to regulate more than 200 genes and to exert a variety of biological effects in almost every tissue in the body, including antiproliferative, pro-apoptotic, pro-differentiation and anti-angiogensis effects in cancer cells *in vivo* and *in vitro*. The clinical use of 1α,25$(OH)_2$D3 is impeded by the lethal side effects of hypercalcemia and hypercalciuria. Therefore, 1α,25$(OH)_2$D3 analogs, which are either equipotent or more potent than 1α,25$(OH)_2$D3 in inhibiting tumor cell growth but with less hypercalcemic and hypercalciuric side effects, have been developed for the treatment of different cancers, and either used alone or in combination with other anticancer agents. The inversed association between vitamin D status and incidence of many forms of cancer has suggested that vitamin D could play a role in the prevention of these types of cancer. Although it is still controversial whether this association exists for pancreatic cancer, biochemical evidence clearly indicates that pancreatic cancer cells are responsive to the inhibitory effect of 1α,25$(OH)_2$D3 and its analogs *in vitro* and *in vivo*. In this chapter, we summarize the recent progress in the epidemiological studies of sunlight, and vitamin D nutritional status, and discuss the biochemical studies using 1α,25$(OH)_2$D3 and its analogs in pancreatic cancer cells with particular emphasis on the molecular mechanism of anti-cancer actions of 1α,25$(OH)_2$D3.

Keywords: analogs, metabolism, apoptosis, differentiation, antiproliferation

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_21, © Wageningen Academic Publishers 2013

Abbreviation

1α,25(OH)$_2$D	1α,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
CYP24A1	25(OH)D-24-hydroxylase
CYP27B1	25(OH)D-1α-hydroxylase
DBP	Vitamin D binding protein
DM	Diabetes mellitus
EGFR	Epidermal growth factor receptor
IPMN	Intrapancreatic mucinous neoplasia
MART-10	19-nor-2α-(3-hydroxypropyl)-1α,25(OH)$_2$D3
MCN	Mucinous cystic neoplasia
miRNA	MicroRNA
PanINs	Pancreatic intraepithelial neoplasia
PCA	Pancreatic adenocarcinoma
UV	Ultraviolet

21.1 Introduction

PCA is one of the most lethal human malignancies. PCA ranks fourth in the Western countries and fifth worldwide among the most common causes of cancer-related mortality (American Cancer Society, 2008). In the US, an estimate of 43,920 new cases of PCA will be identified and 37,390 will die from this disease in 2012 (Siegel *et al.*, 2012). The almost 1:1 ratio of incidence to mortality clearly indicates a poor prognosis and the lethal nature of PCA, due to difficulty of early diagnosis, early local spread, distant metastasis and resistance to traditional chemotherapy and radiotherapy. The overall five-year survival rate is in the range of 1-4%, which is much lower than the other types of cancers, such as colon, breast and prostate cancers (American Cancer Society, 2008). Until the present time, surgical extirpation has been the choice of treatment. However, the overall five-year survival rate after resection is only about 10-29% (Nitecki *et al.*, 1995; Trede *et al.*, 1990; Yeo *et al.*, 1997). Moreover, at the time of presentation, 40% patients already had distant metastasis and another 40% were diagnosed with locally advanced cancer (Haller, 2003), which excluded them from being good candidates for resection.

Although the exact causes of pancreatic cancer remain poorly understood, certain environmental factors have been implicated. The best established and single most important risk factor for pancreatic cancer and the one to clearly avoid is tobacco smoke. Abundant epidemiological studies conducted since 1966 have linked tobacco smoking to an increase in the incidence of pancreatic cancer (Raimondi *et al.*, 2009) and the risk increases with increasing frequency and length of tobacco exposure (Hassan *et al.*, 2007). After cessation of cigarette smoking for 10 years or longer, the risk of pancreatic cancer dwindles (Iodice *et al.*, 2008). Although the association between alcohol and pancreatic cancer is controversial (Jiao *et al.*, 2009; Li Y *et al.*, 2011; Michaud *et al.*, 2010; Rohrmann *et al.*, 2009; Schatzkin and Stolzenberg-Solomon, 2009;

Tramacere *et al.*, 2010), recent evidence has pointed to an association between heavy drinking and pancreatic cancer mortality (Gapstur *et al.*, 2011; Genkinger *et al.*, 2009; Gupta *et al.*, 2010). A pooled analysis of 14 cohort studies reported that in women who consumed more than 30 g of alcohol per day had a positive relationship between pancreatic cancer and alcohol consumption (Genkinger *et al.*, 2009). A prospective study including 1,030,467 US adults 30 years and older reported that consumption of 3 or more drinks per day was associated with pancreatic cancer mortality in never smokers and in ever smokers (Gapstur *et al.*, 2011). Similarly, a population-based case-control study including 532 cases and 1,701 controls in San Francisco Bay Area provides support for heavy alcohol consumption as a risk factor for PCA in men (Gupta *et al.*, 2010). When heavy drinking was combined with heavy smoking, PCA risk increased to 4.3-fold (Talamini *et al.*, 2010). Interestingly, consuming beer or wine had no association with PCA (Gapstur *et al.*, 2011). High caloric intake and obesity were also found to be risk factors for pancreatic cancer (Berrington de Gonzalez *et al.*, 2003; Fryzek *et al.*, 2005; Patel *et al.*, 2005; Reeves *et al.*, 2007), except in Japan (Nitsche *et al.*, 2011). Diets high in processed or red meat, diets low in phytochemicals such as lycopene and flavonols, have been proposed and refuted as risk or protective factors in different trials. Whether fruits and vegetables offer any protective benefit for pancreatic cancer is also conflicting. An earlier large-scaled cohort study failed to show positive benefit of fruits and vegetables (Vrieling *et al.*, 2009). However, a recent case-control study strongly suggests that lower consumption of fruits, vegetables, whole grains, and fiber is associated with a higher pancreatic cancer risk (Jansen *et al.*, 2011). Others, like intake of coffee, use of aspirin and previous cholecystectomy may be contributing factors for pancreatic cancer as well, although less conclusive (Batty *et al.*, 2009; Landi, 2009; Lowenfels and Maisonneuve, 2006). Occupational exposures to chlorinated hydrocarbon solvents, synthetic polymer dust, ionizing radiation, pesticides, diesel and gasoline engine exhaust may also link to pancreatic cancer (Santibanez *et al.*, 2010). For the development of pancreatic cancer, chronic pancreatitis, especially hereditary pancreatitis, is a risk factor (Maisonneuve *et al.*, 2010; Nitsche *et al.*, 2011). DM is also widely considered to be a risk factor for pancreatic adenocarcinoma indepentent of smoking (Li D *et al.*, 2011). Furthermore, several case-control studies suggest that DM could be both an early manifestation and an etiologic factor of pancreatic cancer in males and females (Ben *et al.*, 2011a,b; Maisonneuve *et al.*, 2010).

To search for a potential geographical variation in the incidence of pancreatic cancer, epidemiologists found that the incidence of pancreatic cancer was 3- to 4-times higher in northern latitudes than in areas closer to equator (Curado *et al.*, 2007). Similar variation has been observed previously with prostate, colon and breast cancers (Garland and Garland, 1980; Gorham *et al.*, 1990; Schwartz and Hulka, 1990). This north/south variation has been attributed to sunlight or UV B radiation, which is proportional to vitamin D synthesis in humans, and further suggest that vitamin D deficiency may be a risk factor for pancreatic cancer.

Since pancreatic cancer has poor prognosis due to late diagnosis, and currently available chemotherapy and radiotherapy for pancreatic cancer are relatively ineffective, there is an urgent need to explore new regimens or strategies to prevent and/or treat this disease. In this chapter, we describe an overview of the current advances in the understanding of pancreatic cancer

etiology and development, the pancreatic cancer/vitamin D connection, and the rationales for the potential use of vitamin D and its analogs for the prevention and treatment of pancreatic cancer.

21.2 Pancreatic cancer

21.2.1 Overview

The pancreas, a glandular organ located deep in the abdomen and lay partially behind the stomach and within the concavity of duodenum, is an integral part of the digestive system. The organ has two different functional components, the endocrine and the exocrine parts. The Islets of Langerhans which are the endocrine cells of the pancreas produce and secrete hormones, such as insulin and glucagon, into the bloodstream to regulate the blood glucose levels. The exocrine cells or the Acinar cells produce and excrete digestive enzymes into the duodenum where they assist in the digestion of food. Since the two parts have different normal functions, different kinds of symptoms will occur when tumors arise in either part. However, the vast majority of tumors arise in the exocrine part (Hernández-Muñoz *et al.*, 2008). Cancer of the pancreas is not a single disease (Hruban and Adsay, 2009). In fact, there are as many as twenty different tumors classified under the term 'cancer of the pancreas.' Each of these tumors has a different appearance when examined under a microscope, some require different treatments, and each carries its own unique prognosis. An understanding of the different types of pancreatic tumors is required for rational treatment of this deadly disease (Mahalingam *et al.*, 2009).

21.2.2 The origin and progression of pancreatic adenocarcinoma

PCA originates mainly from the pancreatic ductal epithelium. The disease is believed to evolve from premalignant lesions to invasive cancer, combined with successive accumulation of various gene mutations during the progressive process (Vogelstein and Kinzler, 2004). The premalignant lesions include PanINs, IPMN and MCN, with PanINs being the most common and best characterized histological precursor of pancreatic cancer (Hruban *et al.*, 2008; Takaori, 2007). PanINs are microscopic ductal lesions (less than 5 mm) located in the head of the pancreas, consisting of columnar to mucins-containing cuboidal cells (Hruban *et al.*, 2004). Fourtier classifications are used to describe PanINs, which are PanINs-1A, PanINs-1B (low grade PanINs and refer to as flat and papillary type, respectively), PanINs-2 (intermediate grade PanINs), and PanINs-3 (high grade PanINs), and to reflect its sequentially evolutionary process to PCA. IPMN and MCN are both macroscopic lesions. Current literatures indicate that majority of IPMN may progress into invasive cancer (Delpu *et al.*, 2011). MCNs are large mucin secreting neoplasms and rare. About 20% of MCNs are associated with PCA. Other than originating from premalignant lesions, PCA may derive from a subgroup of about 1-5% of cells with stem cell properties through unlimited self-renewal and asymmetric division (Herrmann *et al.*, 2007; Li *et al.*, 2007). During the progression from low to high grade PanINs, and from precursor lesions to carcinoma, accumulation of gene mutations has been observed, which includes chromosome 7 alterations leading to enhanced expression of EGFR (Korc *et al.*, 1986), up-regulation of the

oncogene KRAS2 (Almoguera *et al.*, 1988) and down-regulation of tumor-suppressor genes, such as CDKN2A (Caldas *et al.*, 1994), DKN2A (Yonezawa *et al.*, 2008), TP53 (Redston *et al.*, 1994), and DPC 4 (Hahn *et al.*, 1996). Among the tumor-suppressor genes, TP53 mutation is the most common single point mutation in human cancer, accounting for up to 75% of PCA (Yonezawa *et al.*, 2008), and is a late event during PanIN multistep progression to adenocarcinoma (Yachida and Iacobuzio-Donahue, 2009).

In addition to various gene mutations, epigenetic alterations have been linked to the etiology of PCA. This kind of alteration is the result of DNA methyltransferase-dependent methylation in CpG islands, histone modification and miRNA expression (Maitra and Hruban, 2008). Sato *et al.* (2003) reported that gene hypomethylation was a frequent epigenetic event in pancreatic cancer and was commonly associated with the overexpression of afftected genes which were not expressed in normal pancreatic tissues. The discovery of miRNA also leads to the finding that numerous miRNAs were aberrantly and differentially expressed in PanINs (Li *et al.*, 2010) and in IPMN lesions (Habbe *et al.*, 2009). These miRNAs have been implicated in the abnormal regulation of cellular differentiation, proliferation, and apoptosis (Maitra and Hruban, 2008). Therefore, it has been suggested that miRNA alterations may contribute to the development of pancreatic cancer and may serve as markers for the early detection of pancreatic neoplasia (Yu *et al.*, 2011)

21.2.3 Therapies for pancreatic cancer

Currently, the standard treatment for resectable pancreatic cancer remains surgery. However, less than 20% of PCA patients are surgically operable (Haller, 2003; http://www.seer.cancer). After operation, adjuvant chemotherapy with either gemcitabine or a combination of 5-fluorouracil and leucovorin is able to increase progression-free period and overall survival (Neoptolemos *et al.*, 2004; Oettle *et al.*, 2007; Regine *et al.*, 2008). Combination of adjuvant chemotherapy and radiation therapy seems to improve overall survival; however, the results are not significant (Herman *et al.*, 2008). For unresectable pancreatic cancer, the principle of treatment is mainly palliative. Historically, 5-fluorouracil was used in spite of poor overall response. Currently, the standard chemotherapy for this group of patients is gemcitamine (Renouf and Moore, 2010). Recently, there have been clinical studies using two new regimens, namely the combination of gemcitabine and erlotinib, or gemcitabine and leucovorin, that demonstrated improvement over gemcitabine alone (Conroy *et al.*, 2011; Moore *et al.*, 2007). Unfortunately, there was a significant increase in toxicity with leucovorin (Oberstein and Wasif Saif, 2011). There are clinical trials underway studying the combination therapy with erlotinib and capecitabine, and the combination of panitumumab with gemcitabine plus erlotinib for dual EGFR inhibition (Oberstein and Wasif Saif, 2011).

21.3 Roles of vitamin D in cancer

21.3.1 History of vitamin D

The discovery of vitamin D is closely associated with the childhood bone disease, rickets. In 1822, Sniadecki, a Polish physician, noted that children living on farms had a lower prevalence of rickets compared to those who lived in the city of Warsaw, Poland. In late 19 century, Theodore Palm, a medical missionary, observed that children living closer to equatorial areas did not have rickets and suggested sunbathing as a possible cure and strategy for rickets prevention. They both attributed their finding of the geographic differences in rickets incidence to varied exposures to sunlight (Mozolowski, 1939; Palm 1890). In 1918, Sir Edward Mellanby successfully raised dogs rachitic by feeding the animals with oats exclusively and keeping them indoors without exposing to sunlight, and was able to cure the rachitic dogs with cod liver oil. During that period, cod liver oil was known to treat night blindness and fracture. Mellanby did not know whether the cure of rickets was due to the newly discovered vitamin A present in cod liver oil (McCollum *et al.*, 1916) or a new substance. It was not until 1922 McCollum clearly demonstrated that the anti-rachitic principle present in cod liver oil was a new substance and named it 'vitamin D' (McCollum *et al.*, 1922). About the same period, Huldshinsky discovered that children with rickets could be cured by exposing to sunlight (Huldshinsky, 1919). Steenbock and Black (1924) then noted that UV-irradiated food could cure rickets, which led to a great discovery later that UV light was capable of transforming one substance stored in food and skin to another form. The findings suggested a close relationship between sunlight exposure and vitamin D. In other words, UV light could produce vitamin D, which possesses anti-rachitic activity.

21.3.2 Sources and metabolism of vitamin D

Vitamin D is rare in food. Therefore, the primary source of vitamin D for humans is the exposure to sunlight in the absence of vitamin D-fortified food and vitamin D supplement. Today, we increasingly depend on vitamin D supplementation to meet our vitamin D requirement due to the concern of UV-induced skin cancers. Vitamin D has two major forms: vitamin D2 and vitamin D3. Vitamin D2 is synthesized from ergosterol found in plants and fungi, whereas vitamin D3 is produced from 7-dehydrocholesterol in animal. When human skin is exposed to UV irradiation (wavelength 290-315 nm), 7-dehydrocholesterol, stored in the basal and suprabasal layers of skin, is photolyzed to form previtamin D3, which is then thermoisomerized to vitamin D3 (Holick *et al.*, 1980). Either vitamin D3 or ingested vitamin D2 enters the blood circulation and is carried by DBP to other organs, including the liver which is the major site of 25-hydroxylation catalyzed by vitamin D-25-hydroxylase (CYP2R1) to form 25(OH)D (Ponchon and DeLuca, 1969; Cheng *et al.*, 2004). Serum 25(OH)D, the index of vitamin D status in humans, has the highest affinity for DBP (Bouillon *et al.*, 1980; Haddad and Walgate, 1976), and naturaly is bound to DBP in circulation. 25(OH)D is then delivered to the kidneys to be hydroxylated by CYP27B1 to form 1α,25$(OH)_2$D, the biologically active form of vitamin D. Both 25(OH)D and 1α,25$(OH)_2$D can be hydroxylated by renal CYP24A1 to form their corresponding 24-hydroxylated metabolites. Hydroxylation at carbon 24 of the vitamin D molecule by CYP24A1 is the first step of inactivation

process for 25(OH)D and 1α,25$(OH)_2$D (Schuster, 2011). However, it is now established that CYP27B1 and CYP24A1 are expressed not only in the kidneys but also in many tissues and cells (Schuster, 2011; Zehnder *et al.*, 2001), including the expression of CYP27B1 in the pancreas (Schwartz *et al.*, 2004). Thus, the pancreas has the ability to activate and inactivate vitamin D in an autocrine/paracrine fashion (Chiang and Chen, 2009).

21.3.3 Functions of vitamin D

The action of 1α,25$(OH)_2$D, the active form of vitamin D, is mediated through its binding to VDR, an endocrine member of the nuclear receptor superfamily (Haussler *et al.*, 1997) to regulate its target genes. The first evidence indicating that vitamin D might have actions beyond regulating calcium and bone metabolism was obtained by an autoradiographic study injecting high specific activity of tritium-labelled 1α,25$(OH)_2$D3 to vitamin D-deficient rats (Stumpf *et al.*, 1979). The authors found that the radioactivity was concentrated not only in the nuclei of small intestine, renal tubules and glomeruli, but also in the nuclei of the epidermis, stomach, pituitary and parathyroid, suggesting that the target cells for 1α,25$(OH)_2$D3 might include those not known for calcium and bone metabolism (Colston *et al.*, 1980). Using a Chip-sequencing assay to define genome-wide mapping of VDR binding, Ramagopalan *et al.* recently reported that VDR was bound to 2,776 genomic sites in 229 vitamin D-regulated genes (Ramagopalan *et al.*, 2010). It is now well-established that 1α,25$(OH)_2$D exhibits anti-proliferative, pro-differentiating, anti-inflammatory, pro-apoptotic activities, inmune regulation and many other functions in a tissue- and cell-specific manner (Adams and Hewison, 2010; Bikle, 2009; Feldman *et al.*, 2008) and, so far, it has been shown to have growth inhibitory effect on prostate, colon, breast, lung, liver and pancreatic cancer cells, which express VDR (Chiang *et al.*, 2011; Colston *et al.*, 1980; Cross *et al.*, 1991; Kawa *et al.*, 1997; Skowronski *et al.*, 1993).

21.3.4 The mechanisms of anti-cancer effects of vitamin D

The mechanism involved in VDR-mediated anti-cancer effects includes (1) cell growth arrest; (2) apoptosis; (3) anti-angiogenesis; (4) pro-differentiation; and (5) anti-inflammation (Adams and Hewison, 2010; Bikle, 2009; Chen and Holick, 2003; Feldman *et al.*, 2008; Welsh, 2011).

Cell growth arrest

Evidence that VDR is required for the antiproliferative effect of 1α,25$(OH)_2$D3 in cancer cell lines has been obtained using stable transfection of cDNA that encodes the VDR into JCA-1 prostate carcinoma cells to increase the VDR concentration (Miller, 1998). This caused proportional increases in antiproliferative effects and CYP24A1 activity in the presence of 1α,25$(OH)_2$D3. Conversely, stable transfection of antisense VDR cDNA to ALVA-31 prostate cancer cells to knockdown VDR (Miller, 1998) attenuated the ability of 1α,25$(OH)_2$D to inhibit cell growth and induce CYP24A1 expression by 1α,25$(OH)_2$D3. Using a different approach, Zinser *et al.* (2003) studied cancer cells derived from VDR null animals and showed that the cells were completely resistant to 1α,25$(OH)_2$D3-mediated growth arrest and apoptosis over the range of 0.01-100

nM 1α,25(OH)$_2$D3. Overall, these data demonstrate that the 1α,25(OH)$_2$D-dependent induction of cell cycle arrest, CYP24A1 upregulation, and apoptosis in cancer cells is dependent on the nuclear VDR. In pancreatic cancer cells, 1α,25(OH)$_2$D3 inhibited their proliferation through cell cycle arrest at the G_0/G_1 phase (Kawa *et al.*, 1996), which in turn is mediated through the up-regulation of p21 and p27, followed by the down-regulation of cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors (Kawa *et al.*, 1997). During the cell cycle progression, E2F-1 transcription factor binds specifically to hypophosphorylated retinoblastoma protein (Powers *et al.*, 2004). In mid to late G_1 phase, cyclin-dependent kinases phosphorylate retinoblastoma protein and displace the E2F-1 transcriptional factor, which further activates the expression of S phase genes required for DNA replication (Krtolica *et al.*, 1998). The activity of cyclin-dependent kinases required for G_1/S transition is regulated by endogenous CDK inhibitors (i.e. p21 and p27). Interestingly, studies using LNCaP prostate cancer cells and HepG2 liver cancer cells have suggested that the up-regulation of p27 proteins induced by 1α,25(OH)$_2$D3 or its analogs may not involve new p27 mRNA synthesis (Luo *et al.*, 2009; Yang and Burnstein, 2003; Yang *et al.*, 2002). In fact, the up-regulation of p27 protein expression is likely the consequence rather than the cause of 1α,25(OH)$_2$D3-induced growth inhibition (Flores *et al.*, 2010). Flores *et al.*(2010) infected LNCaP cells with a retrovirus containing a sh-p27 or sh-Luc (served as control) and found that while p27 was depleted by 74% in sh-p27 infected cells, compared to the cells infected with sh-Luc, the knockdown of p27 had no effect on 1α,25(OH)$_2$D3-induced cell growth inhibition. Thus, it was concluded that p27 up-regulation in LNCaP prostate cancer cells was not essential for 1α,25(OH)$_2$D3-mediated growth inhibition. Taken together, further investigation using pancreatic cancer cells infected with sh-p27 is needed to establish the role of p27 in the antiproliferative effect of 1α,25(OH)$_2$D3.

In addition to the direct effects of 1α,25(OH)$_2$D3 on cell cycle-related genes, the hormone may act indirectly through other mechanisms on these genes. For example, 1α,25(OH)$_2$D3 has been shown to up-regulate the insulin-like growth factor binding protein-3 and transforming growth factor-β signaling pathways and down-regulate the EGFR signaling cascade (Huynh *et al.*,1998; Tong *et al.*, 1999; Yanagisawa *et al.*, 1999). In prostate cells, it has been proposed that the modulation of prostaglandin concentration by 1α,25(OH)$_2$D3 may be one mechanism responsible for the growth inhibitory action of 1α,25(OH)$_2$D3 (Moreno *et al.*, 2005).

Apoptosis

1α,25(OH)$_2$D3 induces apoptosis in a variety of cancer cells to exert anti-tumor effects by repressing the expression of the anti-apoptotic proteins BCL2 and BCL-X_L, or inducing the expression of pro-apoptotic proteins, such as BAX, BAK and BAD (Chen and Holick, 2003). In addition, 1α,25(OH)$_2$D3 may directly activate caspase effector molecules to induce apoptosis. Activation of the caspase cascades, particularly caspase-3, -6, -7 and -9, is mainly induced by the release of cytochrome c from the mitochondrial membrane, into the cytosol. Among them, caspase-3 is the prime inducer of apoptosis (Duan *et al.*, 2003; Gown and Willingham, 2002; Resendes *et al.*, 2004). In our studies with HepG2 cells, no expression of active caspase 3 protein was found either in the control or the group treated with 1α,25(OH)$_2$D3 as measured by western

blot analysis (Chiang *et al.*, 2011). Flow cytometry study with Annexin V-FITC and PI staining to analyze apoptotic and necrotic cell populations of HepG2 cells after 1α,25$(OH)_2$D3 treatment also showed similar apoptotic and necrotic cell populations between the control and the treated groups, suggesting that the decrease in cell number after1α,25$(OH)_2$D3 treatment in HepG2 cells is apoptosis-independent. The 1α,25$(OH)_2$D3-induced apoptotic response may also be mediated by the destabilization of telomerase reverse transcriptase mRNA, that will lead to the down-regulation of telomerase activity (Jiang *et al.*, 2004). Therefore, 1α,25$(OH)_2$D3 induces apoptosis in a cell-specific manner (Chaudhry *et al.*, 2001; Ylikomi *et al.*, 2002) and may involve different mechanisms in different cells.

Anti-angiogenesis

The evidence that vitamin D might have effects on angiogenesis was first suggested by Merke *et al.* (1989), who demonstrated the expression of VDR by immunohistochemical technique using a specific monoclonal antibody against VDR in venular and capillary endothelial cells of human skin biopsies. In their study, they were able to demonstrate CYP27B1 activity and the growth inhibition in the presence of 25(OH)D3 and 1α,25$(OH)_2$D3 in the bovine aortic endothelial cells. Subsequently, various studies showed 1α,25$(OH)_2$D3 inhibited the proliferation of cultured endothelial cells and reduced angiogenesis in animal models (Chung *et al.*, 2006; Iseki *et al.*, 1999; Mantell *et al.*, 2000). 1α,25$(OH)_2$D3 can also up-regulate mRNA levels of the potent anti-angiogenic factor thrombospondin 1 in human colon tumor cells (Fernandez-Garcia *et al.*, 2005). The hormone can also interrupt the angiogenic factor interleukin 8 signaling, leading to the inhibition of endothelial cell migration and tube formation (Bao *et al.*, 2006). It has been proposed that the inhibitory effect of 1α,25$(OH)_2$D3 on metastasis observed in prostate and lung murine models may partially depend on its anti-angiogenic property (Getzenberg *et al.*, 1997; Nakagawa *et al.*, 2005). It should be noted that in certain cells, 1α,25$(OH)_2$D3 may be angiogenic instead of anti-angiogenic, e.g. it induces the angiogenic factor interleukin 8 in SCC cells (Lin *et al.*, 2002) and upregulates VEGF mRNA in vascular smooth muscle cells (Cardus *et al.*, 2006).

Pro-differentiation

The seminal observation by Abe *et al.* (1981) that 1α,25$(OH)_2$D3 was capable of inducing mouse myeloid leukemia cells to differentiate into multinucleated macrophages provides the first evidence that 1α,25$(OH)_2$D3 has functions other than regulating calcium and phosphate homeostasis. Later, it was shown that 1α,25$(OH)_2$D3 can inhibit proliferation and increase the expression of differentiation markers, including involucrin, transglutaminase, loricrin and filaggrin, and enhance cornified envelope formation in cultured keratinocytes (Bikle, 2009). By maintaining the ordered cellular proliferation and differentiated epithelium, 1α,25$(OH)_2$D3 is able to contribute to skin cancer prevention (Bikle, 2009). Now, we know that in cultured cells, administration of 1α,25$(OH)_2$D3 or its analogs can regulate the expression of a variety of genes that are associated with the differentiated cell of origin (Akutsu *et al.*, 2001; Guzey *et al.*, 2004; Palmer, 2003), and thus modulate processes critical for tumor growth and metastasis (Fernandez-Garcia *et al.*, 2005). In SW 480-ADH human colon carcinoma cell line, 1α,25$(OH)_2$D3 induces

differentiation by promoting the expression of proteins implicated in adherent junction formation, including differentiation marker E-cadherin (Palmer *et al.*, 2001). This process is mediated by the up-regulation of Id1gene and down-regulation of Id2 gene in response to 1α,25$(OH)_2$D3 (Fernandez-Garcia *et al.*, 2005). In breast cancer cell lines, the induction of differentiation markers, such as E-cadherin, was also observed following 1α,25$(OH)_2$D3-induced growth arrest (Kemmis *et al.*, 2006; Lazzaro *et al.*, 2000; Pendas-Franco *et al.*, 2008). Thus, 1α,25$(OH)_2$D3-induced differentiation may be one mechanism responsible for inhibiting tumor growth and metastases.

Anti-inflammation

Animal studies have linked the anti-cancer effects of 1α,25$(OH)_2$D3 to its ability to regulate inflammation (Welsh, 2011). In colon carcinoma cells, 1α,25$(OH)_2$D3 is capable of interrupting the wnt-mediated crosstalk between tumor epithelial cells and macrophages in the tumor microenvironment by blocking the production of IL-1β, an inflammatory cytokine produced by tumor-associated macrophages (Kaler *et al.*, 2009). Conversely, any disruption of the 1α,25$(OH)_2$D3/VDR signaling pathway, such as up-regulation of the transcriptional repressor SNAIL, may enhance epithelial cell inflammation and exacerbate colon cancer progression. Feldman and colleagues performed cDNA-microarray analyses of normal and cancer-derived primary prostate epithelial cells (Peehl *et al.*, 2004) and LNCaP cells (Krishnan *et al.*, 2004) and found that 1α,25$(OH)_2$D3 regulated a wide array of genes, including those involved in the synthesis and catabolism of prostaglandins, well-established inflammatory compounds. They showed that 1α,25$(OH)_2$D3 up-regulated the expression of NAD+-dependent 15-hydroxy-prostaglandin dehydrogenase gene and down-regulated cyclooxygenase-2 (COX-2) expression. Since prostaglandins are known to play a role in the development and progression of many cancers (Mantovani *et al.*, 2008), the ability of 1α,25$(OH)_2$D3 to decrease prostaglandin concentration strongly suggests that one mechanism of anti-cancer effect of vitamin D may be mediated through its anti-inflammatory action.

21.4 Vitamin D and pancreatic cancer

21.4.1 Overview

Vitamin D deficiency has been implicated in many chronic diseases and cancers (Garland *et al.*, 2009; Holick, 2007). Regarding pancreatic cancer, laboratory studies have firmly established the anti-tumor effects of 1α,25$(OH)_2$D3 in cultured pancreatic cancer cells and in animal models. However, the epidemiological data are conflicting. This could be due to the aggressive nature of this disease, and the lack of a 'zero' or 'near zero' lag time blood drawing between diagnosis and serum 25(OH)D measurement that is critical to demonstrate the inverse relationship between the incidence of pancreatic disease and vitamin D status (Mohr *et al.*, 2010).

21.4.2 Biochemical evidence

As mentioned in Sections 21.3.3 and 21.3.4, 1α,25$(OH)_2$D3 possesses anti-tumor activity through anti-proliferative, pro-apoptotic, and pro-differentiation actions in a cell- and tissue-specific manner (Adams and Hewison, 2010; Bikle, 2009; Chen and Holick, 2003; Feldman *et al.*, 2008; Plum and DeLuca, 2010). Regarding pancreatic cancer, 1α,25$(OH)_2$D3 has been shown to up-regulate the expression of p21 and p27 and down-regulate the expression of cyclins A, D1, and E, leading to cell cycle arrest at G_0/G_1 phase (Kawa *et al.*, 1997). However, 1α,25$(OH)_2$D3 is known to cause hypercalcemia and hypercalciuria side effects. To overcome these lethal side effects caused by systemic administration of 1α,25$(OH)_2$D3, numerous less calcemic or noncalcemic analogs of 1α,25$(OH)_2$D3 have been synthesized and studied *in vitro* and *in vivo* animal models. Some of them have been found to have more potent anti-tumor activities mediated by promoting cell-cycle arrest, cellular differentiation, and/or apoptosis in pancreatic cancer cells *in vitro* and in the xenograft animal model (Colston *et al.*, 1997; Kawa *et al.*, 1996, 1997; Pettersson *et al.*, 2000; Schwartz *et al.*, 2008; Zugmaier *et al.*, 1996). One of these analogs, EB-1089, has been investigated in a phase II clinical trial to treat advanced pancreatic cancer. However, the trial showed that the analog failed to significantly prolong the survival of patients (Evans *et al.*, 2002). In a more recent published phase II clinical trial enrolling 25 advanced pancreatic cancer patients, a combination of oral 1α,25$(OH)_2$D3 (0.5 μg/kg) and docetaxel significantly increased the period of time-to-progress of pancreatic cancer as compared to treatment with docetaxel alone (Blanke *et al.*, 2009). A VDR-alkylating derivative of 1α,25$(OH)_2$D3, 1α,25-dihydroxyvitamin D3-3-bromoacetate, has been studied *in vitro* and was shown to inhibit the growth of several pancreatic cancer cell lines to a greater extent than 1α,25$(OH)_2$D3 (Persons *et al.*, 2010). The *in vitro* activity was further enhanced by combining with 5-amino-imidazole-4-carboxamide-1-beta-4-ribofuranoside (Persons *et al.*, 2010). In our laboratory, a new vitamin D analog, MART-10 has been shown to be about 1000-fold more active than 1α,25$(OH)_2$D3 in inhibiting the proliferation of LNCaP and PC-3 prostate cancer cell lines and HepG2 liver cancer cell line *in vitro* (Chen *et al.*, 2007; Chiang *et al.*, 2011; Flanangan *et al.*, 2009; Iglesias-Gato *et al.*, 2011). Most importantly, MART-10 does not increase serum calcium in animals (Iglesias-Gato *et al.*, 2011). Furthermore, MART-10 is more resistant to CYP24A1-mediated degradation pathway and has a lower binding affinity for DBP compared to 1α,25$(OH)_2$D3, suggesting that this analog would be more bioavailable than 1α,25$(OH)_2$D3 in circulation (Flanangan *et al.*, 2009; Iglesias-Gato *et al.*, 2011). Given the poor prognosis and little effective therapeutive options against pancreatic cancer, these two new analogs are promising candidates for further pre-clinical studies, and subsequent clinical trials for pancreatic cancer patients.

21.4.3 Ecological and epidemiological studies of vitamin D and pancreatic cancer

Vitamin D status has been shown to possess positive impacts on the incidence of prostate, colon and breast cancers in a number of ecological and epidemiological studies (Garland and Garland, 1980; Gorham *et al.*, 1990; Schwartz and Hulka, 1990). For pancreatic cancer, two earlier epidemiologic studies reported inconsistent relationship between pancreatic cancer incidence and vitamin D status (Skinner *et al.*, 2006; Stolzenberg-Solomon *et al.*, 2006). A later nested case-

control study conducted by Stolzenberg-Solomon *et al.* (2009) again failed to support the inversed association between the circulating concentration of 25(OH)D and the risk of pancreatic cancer. The same group further reported that a high 25(OH)D level exceeding 100 nmol/l (40 ng/ml) would have a 2-fold increase in pancreatic cancer incidence (odds ratio = 2.12, 95% confidence interval: 1.23, 3.64) (Stolzenberg-Solomon *et al.*, 2010). On the contrary, Mohr *et al.* (2010) reported that pancreatic cancer incidence rates were half as high in countries with estimated serum 25(OH)D >30 ng/ml than in those with ≤30 ng/ml. A recent report by Wolpin *et al.* (2012) demonstrated that among participants in a pooled analysis of nested case-control studies with 451 cases and 1,167 controls from five large prospective cohorts with a follow-up ranging from 12.2 yr in Women's Health Initiative-Observational Study to 25.3 yr in Physicians's Health Study, higher plasma levels of 25(OH)D were associated with a lower risk of pancreatic cancer. The study further implies that low circulating levels of 25(OH)D may predispose individuals to the development of pancreatic cancer. No increased risk was found in subjects with 25(OH)D ≥100 nmol/l as reported in a previous study by Stolzenberg-Solomon *et al.* (2010). The lack of association between serum 25(OH)D levels and pancreatic cancer risk in some studies could be due to that the studies utilized a single serum sample obtained years prior to diagnosis for 25(OH)D measurement. It may well be that serum 25(OH)D changed over the years after the measurement. For example, Yin *et al.* (2010) conducted case-control studies with zero lag time between diagnosis and serum 25(OH)D measurement, not nest studies, and found an inversed correlation between serum 25(OH)D level and breast cancer.

Interestingly, the inversed relation between sunlight exposure and pancreatic cancer has been more consistent. For example, the death rate of pancreatic cancer has been found to be inversely associated with sun exposure (Boscoe and Schymura, 2006; Grant, 2007; Stolzenberg-Solomon *et al.*, 2006; Tuohimaa *et al.*, 2007). Stolzenberg-Solomon *et al.* (2009) did confirm a positive association among subjects with low estimated annual residential solar UVB exposure and pancreatic cancer risk in the same study which did not show an inversed relation between serum 25(OH)D levels and pancreatic cancer risk. Mohr *et al.* (2010) as well as many other studies (Giovannucci *et al.*, 2006; Kato *et al.*, 1985; Neale *et al.*, 2009) also found an inversed association between UVB irradiation and incidence rates of pancreatic cancer worldwide.

Results from investigating association of insulin and glucose levels and the development of pancreatic cancer have also indicated a positive association between high insulin and glucose levels and pancreatic cancer (Henning *et al.*, 2004; Huxley *et al.*, 2005; Michaud *et al.*, 2007; Stolzenberg-Solomon *et al.*, 2005). Since vitamin D is capable of regulating the synthesis, binding and actions of insulin (Maestro *et al.*, 2000, 2003; Mathieu *et al.*, 2005), the finding may imply that an inverse relationship between pancreatic cancer incidence and vitamin D status could very well exist.

To resolve the question whether vitamin D status has a preventive benefit against the development of pancreatic cancer, more careful selection of study population according to VDR genotypes and possibly the genotypes of DBP and CYP enzymes involved in vitamin D metabolism, such as CYP24A1, CYP27B1 and CYP2R1, may be essential. Several recent genome-wide association

studies have suggested that variation in vitamin D pathway genes may affect serum concentrations of 25(OH)D (Ahn *et al.*, 2010; McGrath *et al.*, 2010; Signorello *et al.*, 2011; Wang *et al.*, 2010).

21.5 Conclusions

Pancreatic adenocarcinoma remains to be a lethal human malignancy without effective treatments at the present time. During the past few years major advances have been made in the understanding of the molecular mechanisms underlying the initiation and progression of pancreatic cancer, mainly through the efforts of geome- and epigenome-wide screening techniques. By knowing the earliest molecular signatures of carcinogenesis, we may be able to either prevent its initiation, detect the precursor lesions at the early stages or before the tumor cells metastasize to other organs. The active form of vitamin D3, 1α,25$(OH)_2$D3, has been recognized as one of the most potent compounds in inhibiting cell proliferation and inducing cellular differentiation of normal and cancer cells for more than 30 years, including pancreatic cells. Recently, a genome-wide mapping of VDR binding study using a Chip-sequencing assay indicates that there are 2,776 genomic binding sites for VDR in 229 vitamin D-regulated genes. Coupled with this information, genomic profiling has been used to gain insight into the anti-tumor mechanisms of vitamin D. We now know that the anti-tumor actions of 1α,25$(OH)_2$D and its analogs may include pro-apoptosis, anti-angiogenesis, anti-inflammatory in addition to anti-proliferation and pro-differentiation. However, at the present time we still don't know whether some of the vitamin D-affected genes can prevent the normal or precancerous cells from becoming cancerous. As vitamin D deficiency is implicated with the increased incidence of many cancers and other chronic diseases, the likelihood that this kind of 'prevention' genes is present is very high. Regarding the treatment of advanced pancreatic cancer, a phase II trial using a combination of 1α,25$(OH)_2$D and docetaxel showed a significant increase in the period of time-to-progress of pancreatic cancer as compared to docetaxel alone. In our laboratory, we have studied an analog of 1α,25$(OH)_2$D3, MART-10, and demonstrate that this analog is non-calcemic, and is much more potent and more bioavailable than 1α,25$(OH)_2$D3 in prostate and liver cancer cells. Knowing the properties of this analog, we may be able to use this analog in combination with docetaxel or other chemotherapeutic agents to treat advanced pancreatic adenocarcinoma patients. In conclusion, from the current biochemical, epidemiological and ecological evidence accumulated for the past several decades, sufficient vitamin D nutrition may be beneficial for the prevention of cancers, including pancreatic adenocarcinoma, and the non-calcemic and highly potent 1α,25$(OH)_2$D, such as MART-10, in combination with other chemotherapeutic agents could be a promising regimen for treating advanced pancreatic cancer.

References

Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshiki, S. and Suda, T., 1981. Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D3. Proc Natl Acad Sci USA 78, 4990-4994.

Adams, J.S. and Hewison, M., 2010. Update in vitamin D. J Clin Endocrinol Metab 95, 471-478.

Akutsu, N., Lin, R., Bastien, Y., Bestawros, A., Enepekides, D.J., Black, M.J. and White, J.H., 2001. Regulation of gene expression by 1alpha,25-dihydroxyvitamin D3 and its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells.Mol Endocrinol 15, 1127-1139.

Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N. and Perucho, M., 1988. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 53, 549-554.

American Cancer Society, 2008. Cancer facts and figures. American Cancer society, Atlanta, GA, USA.

Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K.C., McCullough, M.L., Gallicchio, L., Jacobs, E.J., Ascherio, A., Helzlsouer, K., Jacobs, K.B., Li, Q., Weinstein, S.J., Purdue, M., Virtamo, J., Horst, R., Wheeler, W., Chanock, S., Hunter, D.J., Hayes, R.B., Kraft, P. and Albanes, D., 2010. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 19, 2739-2745.

Batty, G.D., Kivimaki, M., Morrison, D., Huxley, R., Smith, G.D., Clarke, R., Marmot, M.G. and Shipley, M.J., 2009. Risk factors for pancreatic cancer mortality: extended follow-up of the original Whitehall Study. Cancer Epidemiol Biomarkers Prev 18, 673-675.

Bao, B.Y., Yeh, S.D. and Lee, Y.F., 2006. 1alpha,25-dihydroxyvitamin D3 suppresses interleukin-8- mediated prostate cancer cell angiogenesis.s. Carcinogenesis 27, 1883-1893.

Ben, Q., Cai, Q., Li, Z., Yuan, Y., Ning, X., Deng, S. and Wang, K., 2011a. The relationship between new-onset diabetes mellitus and pancreatic cancer risk: a case-control study. Eur J Cancer 47, 248-254.

Ben, Q., Xu, M., Ning, X., Liu, J., Hong, S., Huang, W., Zhang, H. and Li, Z., 2011b. Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies. Eur J Cancer 47, 1928-1937.

Berrington de Gonzalez, A., Sweetland, S. and Spencer, E., 2003. A meta-analysis of obesity and the risk of pancreatic cancer. Br J Cancer 89, 519-523.

Bikle, D., 2009. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 94, 26-34.

Blanke, C.D., Beer, T.M., Todd, K., Mori, M., Stone, M. and Lopez, C., 2009. Phase II study of calcitriol-enhanced docetaxel in patients with previously untreated metastatic or locally advanced pancreatic cancer. Invest New Drugs 27, 374-378.

Boscoe, F.P. and Schymura, M.J., 2006. Solar ultraviolet-B exposure and cancer incidence and mortality in the United States, 1993-2002. BMC Cancer 6, 264.

Bouillon, R., Van Baelen, H., Tan, B.K. and De Moor, P., 1980. The isolation and characterization of the 25-hydroxyvitamin D-binding protein from chick serum. J Biol Chem 255, 10925-10930.

Caldas, C., Hahn, S.A., da Costa, L.T., Redston, M.S., Schutte, M., Seymour, A.B., Weinstein, C.L., Hruban, R.H., Yeo, C.J. and Kern, S.E., 1994. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat. Gene, 8, 27-32. Erratum in: Nat. Genet 8, 410.

Cardús, A., Parisi, E., Gallego, C., Aldea, M., Fernández, E. and Valdivielso, J.M., 2006. 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. Kidney Int 69, 1377-1384.

Chaudhry, M., Sundaram, S., Gennings, C., Carter, H. and Gewirtz, D.A., 2001. The vitamin D3 analog, ILX-23-7553, enhances the response to adriamycin and irradiation in MCF-7 breast tumor cells. Cancer Chemother Pharmacol 47, 429-436.

Chen, T.C. and Holick, M.F., 2003. Vitamin D and prostate cancer prevention and treatment. Trends Endocrinol Metab 144, 423-430.

Chen, T.C., Persons, K.S., Zheng, S., Mathieu, J., Holick, M.F., Lee, Y.F., Bao, B., Arai, M.A. and Kittaka, A., 2007. Evaluation of C-2-substituted 19-nor-1α,25-dihydroxyvitamin D3 analogs as therapeutic agents for prostate cancer. J Steroid Biochem Mol Biol 103, 717-720.

Cheng, J.B., Levine, M.A., Bell, N.H., Mangelsdorf, D.J. and Russell, D.W., 2004. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 101, 7711-7715.

Chiang, K.C. and Chen, T.C., 2009. Vitamin D for the prevention and treatment of pancreatic cancer. World J Gastroenterol 15, 3349-3354.

Chiang, K.C., Yeh, C.N., Chen, H.Y., Lee, J.M., Juang, H.H., Chen, M.F., Takano, M., Kittaka, A. and Chen, T.C., 2011. 19-Nor-2α-(3-hydroxypropyl)-1α,25-dihydroxyvitamin D3 (MART-10) is a potent cell growth regulator with enhanced chemotherapeutic potency in liver cancer cells. Steroids 76, 1513-1519.

Chung, I., Wong, M.K., Flynn, G., Yu, W.D., Johnson, C.S. and Trump, D.L., 2006. Differential antiproliferative effects of calcitriol on tumor-derived and matrigel-derived endothelial cells. Cancer Res 66, 8565-8573, 1513-1519.

Colston, K., Hirt, M. and Feldman, D., 1980. Organ distribution of the cytoplasmic 1,25-dihydroxycholecalciferol receptor in various mouse tissues. Endocrinology 107, 1916-1922.

Colston, K.W., James, S.Y., Ofori-Kuragu, E.A., Binderup, L. and Grant, A.G., 1997. Vitamin D receptors and anti-proliferative effects of vitamin D derivatives in human pancreatic carcinoma cells *in vivo* and *in vitro*. Br J Cancer 76, 1017-1020.

Conroy, T., Desseigne, F., Ychou, M., Bouché, O., Guimbaud, R., Bécouarn, Y., Adenis, A., Raoul, J.L., Gourgou-Bourgade, S., de la Fouchardière, C., Bennouna, J., Bachet, J.B., Khemissa-Akouz, F., Péré-Vergé, D., Delbaldo, C., Assenat, E., Chauffert, B., Michel, P., Montoto-Grillot, C., Ducreux, M., Groupe Tumeurs Digestives of Unicancer and the PRODIGE Intergroup, 2011. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 364, 1817-1825.

Cross, H.S., Huber, C. and Peterlik, M., 1991. Antiproliferative effect of 1,25-dihydroxyvitamin D3 and its analogs on human colon adenocarcinoma cells (CaCo-2):influence of extracellar calcium. Biochem Biophys Res Commun 179, 57-62.

Curado, M.P., Edward, B.K., Shin, H.R.and Storm, H., 2007. Cancer Incidence in Five Continents. Vol IX. IARC Scientific Pub. No.160. IARC, Lyon, France.

Delpu, Y., Hanoun, N., Lulka, H., Sicard, F., Selves, J., Buscail, L., Torrisani, J. and Cordelier, P., 2011. Genetic and epigenetic alterations in pancreatic carcinogenesis. Curr Genomics 12, 15-24.

Duan, W.R., Garner, D.S., Williams, S.D., Funckes-Shippy, C.L.,Spath, I.S. and Blomme,E.A., 2003. Comparison of immunohistochemistry for activated caspase-3 and cleaved cytokeratin 18 with the TUNEL method for quantification of apoptosis in histological sections of PC-3 subcutaneous xenografts. J Pathol 199, 221-228.

Evans, T.R., Colston, K.W., Lofts, F.J., Cunningham, D., Anthoney, D.A., Gogas, H., de Bono, J.S., Hamberg, K.J., Skov, T. and Mansi, J.L., 2002. A phase II trial of the vitamin D analogue Seocalcitol (EB1089) in patients with inoperable pancreatic cancer. Br J Cancer 86, 680-685.

Feldman, D., Malloy, P.J., Krishnan, A.V. and Balint, E., 2008. Vitamin D: biology, action, and clinical implications. In: Marcus, R., Feldman, D., Nelson, D.A. and Rosen, C.J. (eds.) Osteoporosis, 3rd Edition. Academic Press, San Diego, CA, USA, Vol. 1, pp. 317-382.

Fernandez-Garcia, N.I., Palmer, H.G., Garcia, M., Gonzalez-Martin, A., del Rio, M., Barettino, D., Volpert, O., Muñoz, A. and Jimenez, B., 2005. 1alpha,25-Dihydroxyvitamin D3 regulates the expression of Id1 and Id2 genes and the angiogenic phenotype of human colon carcinoma cells. Oncogene 24, 6533-6544.

Flanagan, J.N., Zheng, S., Chiang, K.C., Kittaka, A., Sakaki, T., Nakabayashi, S., Zhao, X., Spanjaard, R.A., Persons, K.S., Mathieu, J.S., Holick, M.F. and Chen, T.C., 2009. Evaluation of 19-nor-2alpha-(3-hydroxypropyl)-1alpha,25-dihydroxyvitamin D3 as a therapeutic agent for androgen-dependent prostate cancer. Anticancer Res 29, 3547-3553.

Flores, O., Wang, Z., Knudsen, K.E. and Burnstein, K.L., 2010. Nuclear targeting of cyclin-dependent kinase 2 reveals essential roles of cyclin-dependent kinase 2 localization and cyclin E in vitamin D-mediated growth inhibition. Endocrinology 151, 896-908.

Fryzek, J.P., Schenk, M., Kinnard, M., Greenson, J.K. and Garabrant, D.H., 2005. The association of body mass index and pancreatic cancer in residents of southeastern Michigan, 1996-1999. Am J Epidemiol 162, 222-228.

Garland, C.F. and Garland, F.C., 1980. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol 9, 227-231.

Garland, C.F., Gorham, E.D., Mohr, S.B. and Garland, F.C., 2009. Vitamin D for cancer prevention: global perspective. Ann Epidemiol 19, 468-483.

Gapstur, S.M., Jacobs, E.J., Deka, A., McCullough, M.L., Patel, A.V. and Thun, M.J., 2011. Association of alcohol intake with pancreatic cancer mortality in never smokers. Arch Intern Med 171, 444-451.

Genkinger, J.M., Spiegelman, D., Anderson, K.E., Bergkvist, L., Bernstein, L., van den Brandt, P.A., English, D.R., Freudenheim, J.L., Fuchs, C.S., Giles, G.G., Giovannucci, E., Hankinson, S.E., Horn-Ross, P.L., Leitzmann, M., Männistö, S., Marshall, J.R., McCullough, M.L., Miller, A.B., Reding, D.J., Robien, K., Rohan, T.E., Schatzkin, A., Stevens, V.L., Stolzenberg-Solomon, R.Z., Verhage, B.A., Wolk, A., Ziegler, R.G. and Smith-Warner, S.A., 2009. Alcohol intake and pancreatic cancer risk: a pooled analysis of fourteen cohort studies. Cancer Epidemiol Biomarkers Prev 18, 765-776.

Getzenberg, R.H., Light, B.W., Lapco, P.E., Konety, B.R., Nangia, A.K., Acierno, J.S., Dhir, R., Shurin, Z., Day, R.S., Trump, D.L. and Johnson, C.S., 1997. Vitamin D inhibition of prostate adenocarcinoma growth and metastasis in the Dunning rat prostate model system. Urology 50, 999-1006.

Giovannucci, E., Liu, Y., Rimm, E.B., Hollis, B.W., Fuchs, C.S., Stampfer, M.J. and Willett, W.C., 2006. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 98, 451-459.

Gorham, E.D., Garland, F.C. and Garland, C.F., 1990. Sunlight and breast cancer incidence in the USSR. Int J Epidemiol 19, 820-824.

Gown, A.M. and Willingham, M.C., 2002. Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase 3. J Histochem Cytochem 50, 449-454.

Grant, W.B., 2007. An ecologic study of cancer mortality rates in Spain with respect to indices of solar UVB irradiance and smoking. Int J Cancer 120, 1123-1128.

Gupta, S., Wang, F., Holly, E.A. and Bracci, P.M., 2010. Risk of pancreatic cancer by alcohol dose, duration, and pattern of consumption, including binge drinking: a population-based study. Cancer Causes Control 21, 1047-1059.

Guzey, M., Luo, J. and Getzenberg, R.H., 2004. Vitamin D3 modulated gene expression patterns in human primary normal and cancer prostate cells. J Cell Biochem 93, 271-285.

Habbe, N., Koorstra, J.B., Mendell, J.T., Offerhaus, G.J., Ryu, J.K., Feldmann, G., Mullendore, M.E., Goggins, M.G., Hong, S.M. and Maitra, A., 2009. MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. Cancer Biol Ther 8, 340-346.

Haddad Jr., J.G. and Walgate, J., 1976. 25-hydroxyvitamin D transport in human plasma. Isolation and partial characterization of calcifidiol-binding protein. J Biol Chem 251, 4803-4809.

Hahn, S.A., Schutte, M., Hoque, A.T., Moskaluk, C.A., da Costa, L.T., Rozenblum, E., Weinstein, C.L., Fischer, A., Yeo, C.J., Hruban, R.H. and Kern, S.E., 1996. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 271, 350-353.

Haller, D.G., 2003. New perspectives in the management of pancreas cancer. Semin Oncol 30, 3-10.

Hassan, M.M., Bondy, M.L., Wolff, R.A., Abbruzzese, J.L., Vauthey, J.N., Pisters, P.W., Evans, D.B., Khan, R., Chou, T.H., Lenzi, R., Jiao, L. and Li, D., 2007. Risk factors for pancreatic cancer: case-control study. Am J Gastroenterol 102, 2696-2707.

Haussler, M.R., Haussler, C.A., Jurutka, P.W., Thompson, P.D., Hsieh, J.C., Remus, L.S., Selznick, S.H. and Whitfield, G.K., 1997. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. J Endocrinol 154 Suppl, S57-73.

Hennig, R., Ding, X.Z. and Adrian, T.E., 2004. On the role of the islets of Langerhans in pancreatic cancer. Histol Histopathol 19, 999-1011.

Herman, J.M., Swartz, M.J, Hsu, C.C., Winter, J., Pawlik, T.M., Sugar, E., Robinson, R., Laheru, D.A., Jaffee, E., Hruban, R.H., Campbell, K.A., Wolfgang, C.L., Asrari, F., Donehower, R., Hidalgo, M., Diaz, L.A. Jr, Yeo, C., Cameron, J.L., Schulick, R.D. and Abrams, R., 2008. Analysis of fluorouracil-based adjuvant chemotherapy and radiation after pancreaticoduodenectomy for ductal adenocarcinoma of the pancreas: results of a large, prospectively collected database at the Johns Hopkins Hospital. J Clin Oncol 26, 3503-3510.

Hernández-Muñoz, I., Skoudy, A., Real, F.X. and Navarro, P., 2008. Pancreatic ductal adenocarcinoma: cellular origin, signaling pathways and stroma contribution. Pancreatology 8, 462-469.

Herrmann, C., Abel, U., Stremmel, W., Jaeger, D. and Herrmann, T., 2007. Short time to progression under first-line chemotherapy is a negative prognostic factor for time to progression and residual survival under second-line chemotherapy in advanced pancreatic cancer. Oncology 73(5-6), 335-339.

Holick, M.F., 2007. Vitamin D deficiency. N Engl J Med 357, 266-281.

Holick, M.F., MacLaughlin, J.A., Clark, M.B., Holick, S.A., Potts, J.T. Jr, Anderson, R.R., Blank, I.H., Parrish, J.A. and Elias, P., 1980. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science, 210, 203-205.

Hruban, R.H. and Adsay, N.V., 2009. Molecular classification of neoplasms of the pancreas. Hum Pathol 612-623.

Hruban, R.H., Maitra, A. and Goggins, M., 2008. Update on pancreatic intraepithelial neoplasia. Int J Clin Exp Pathol 1, 306-316.

Hruban, R.H., Takaori, K., Klimstra, D.S., Adsay, N.V., Albores-Saavedra, J., Biankin, A.V., Biankin, S.A., Compton, C., Fukushima, N., Furukawa, T., Goggins, M., Kato, Y., Klöppel, G., Longnecker, D.S., Lüttges, J., Maitra, A., Offerhaus, G.J., Shimizu, M. and Yonezawa, S., 2004. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol 28, 977-987.

Huldshinsky, K., 1919. Heilung von rachitis durch kunstlich hohensonne. Deut Med Wochenschr 45, 712-713.

Huxley, R., Ansary-Moghaddam, A., Berrington de Gonzalez, A., Barzi, F. and Woodward, M., 2005. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer 92, 2076-2083.

Huynh, H., Pollak, M. and Zhang, J.C., 1998. Regulation of insulin-like growth factor (IGF)II and IGF binding protein 3 autocrine loop in human PC-3 prostate cancer cells by vitamin D metabolite 1α,25(OH)$_2$D3 and its analog EB1089. Int J Oncol 13, 137-143.

Iglesias-Gato, D., Zheng, S., Flanagan, J.N., Jiang, L., Kittaka, A., Sakaki, T., Yamamoto, K., Itoh, T., Lebrasseur, N.K., Norstedt, G. and Chen, T.C., 2011. Substitution at carbon 2 of 19-nor-1α,25-dihydroxyvitamin D(3) with 3-hydroxypropyl group generates an analogue with enhanced chemotherapeutic potency in PC-3 prostate cancer cells. J Steroid Biochem Mol Biol 127, 269-275.

Iodice, S., Gandini, S., Maisonneuve, P. and Lowenfels, A.B., 2008. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. Langenbecks Arch Surg 393, 535-45.

Iseki, K., Tatsuta, M., Uehara, H., Iishi, H., Yano, H., Sakai, N. and Ishiguro, S., 1999. Inhibition of angiogenesis as a mechanism for inhibition by 1alpha-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 of colon carcinogenesis induced by azoxymethane in Wistar rats. Int J Cancer 81, 730-733.

Jansen, R.J., Robinson, D.P., Stolzenberg-Solomon, R.Z., Bamlet, W.R., de Andrade, M., Oberg, A.L., Hammer, T.J., Rabe, K.G., Anderson, K.E., Olson, J.E., Sinha, R. and Petersen, G.M., 2011. Fruit and vegetable consumption is inversely associated with having pancreatic cancer. Cancer Causes Control 22, 1613-1625.

Jiang, F., Bao, J., Li, P., Nicosia, S.V. and Bai, W., 2004. Induction of ovarian cancer cell apoptosis by 1α,25-dihydroxyvitaminD3 through the down-regulation of telomerase. J Biol Chem 279, 53213-53221.

Jiao, L., Silverman, D.T., Schairer, C., Thiebaut, A.C., Hollenbeck, A.R., Leitzmann, M.F., Schatzkin, A. and Stolzenberg-Solomon, R.Z., 2009. Alcohol use and risk of pancreatic cancer: the NIH-AARP Diet and Health Study. Am J Epidemiol 169, 1043-1051.

Kaler, P., Augenlicht, L. and Klampfer, L., 2009. Macrophage-derived IL-1beta stimulates Wnt signaling and growth of colon cancer cells: a crosstalk interrupted by vitamin D3. Oncogene 28, 3892-3902.

Kato, I., Tajima, K., Kuroishi, T. and Tominaga, S., 1985. Latitude and pancreatic cancer. Jpn J Clin Oncol 15, 403-413.

Kawa, S., Nikaido, T., Aoki, Y., Zhai, Y., Kumagai, T., Furihata, K., Fujii, S. and Kiyosawa, K., 1997. Vitamin D analogues up-regulate p21 and p27 during growth inhibition of pancreatic cancer cell lines. Br J Cancer 76, 884-889.

Kawa, S., Yoshizawa, K., Tokoo, M., Imai, H., Oguchi, H., Kiyosawa, K., Homma, T., Nikaido, T. and Furihata, K., 1996. Inhibitory effect of 22-oxa-1,25-dihydroxyvitamin D3 on the proliferation of pancreatic cancer cell lines. Gastroenterology 110, 1605-1613.

Kemmis, C.M.,Salvador, S.M., Smith, K.M. and Welsh, J., 2006. Human mammary epithelial cells express CYP27B1 and are growth inhibited by 25-hydroxyvitamin D-3, the major circulating form of vitamin D-3. J Nutr 136, 887-892.

Korc, M., Meltzer, P. and Trent, J., 1986. Enhanced expression of epidermal growth factor receptor correlates with alterations of chromosome 7 in human pancreatic cancer. Proc Natl Acad Sci USA 83, 5141-5144.

Krishnan, A.V., Shinghal, R., Raghavachari, N., Brooks, J.D., Peehl, D.M. and Feldman, D., 2004. Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. Prostate 59, 243-251.

Krtolica, A., Kruche, N.A. and Ludlow, J.W., 1998. Hypoxia-induced pRB hypophosphorylation results from downregulation of CDK and upregulation of PP1 activities. Oncogene 17, 2295-304.

Lazzaro, G,. Agadir, A., Qing, W., Poria, M., Mehta, R.R., Moriarty, R.M., Das Gupta, T.K., Zhang, X.K. and Mehta, R.G., 2000. Induction of differentiation by 1alpha-hydroxyvitamin D(5) in T47D human breast cancer cells and its interaction with vitamin D receptors. Eur J Cancer 36, 780-786.

Landi, S., 2009. Genetic predisposition and environmental risk factors to pancreatic cancer: A review of the literature. Mutat Res 681(2-3), 299-307.

Li, A., Omuran, N., Hong, S.M., Vincent, A., Walter, K., Griffith, M., Borges, M. and Goggins, M., 2010. Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels. Cancer Res 70, 5226-5237.

Li, C., Heidt, D.G., Dalerba, P., Burant, C.F., Zhang, L., Adsay, V., Wicha, M., Clarke, M.F. and Simeone, D.M., 2007. Identification of pancreatic cancer stem cells. Cancer Res 67, 1030-1037.

Li, D., Tang, H., Hassan, M.M., Holly, E.A., Bracci, P.M., and Silverman, D.T., 2011. Diabetes and risk of pancreatic cancer: a pooled analysis of three large case-control studies. Cancer Causes Control 22, 189-197.

Li, Y., Yang, H. and Cao, J., 2011 Association between alcohol consumption and cancers in the Chinese population – a systematic review and meta-analysis. PLoS One 6, e18776.

Lin, R., Nagai, Y., Sladek, R., Bastien, Y., Ho, J., Petrecca, K., Sotiropoulou, G., Diamandis, E.P., Hudson, T.J. and White, J.H., 2002. Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol 16, 1243-1256.

Lowenfels, A.B. and Maisonneuve, P., 2006. Epidemiology and risk factors for pancreatic cancer. Best Pract Res Clin Gastroenterol 20, 197-209.

Luo, W., Chen, Y., Liu, M., Du, K., Zheng, G., Cai, T., Zhang, W., Zhao, F., Yao, T., Yang, R. and Chen, J., 2009. EB1089 induces Skp2-dependent p27 accumulation, leading to cell growth inhibition and cell cycle G1 phase arrest in human hepatoma cells. Cancer Invest 27, 29-37.

Maestro, B., Campion, J., Davila, N. and Calle, C., 2000. Stimulation by 1,25-dihydroxyvitamin D3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells. Endocr J 47, 383-391.

Maestro, B., Davila, N., Carranza, M.C. and Calle, C., 2003. Identification of a Vitamin D response element in the human insulin receptor gene promoter. J Steroid Biochem Mol Biol 84, 223-230.

Mahalingam, D., Kelly, K.R., Swords, R.T., Carew, J., Nawrocki, S.T. and Giles, F.J., 2009. Emerging drugs in the treatment of pancreatic cancer. Expert Opin Emerg Drugs 14, 311-328.

Maisonneuve, P., Lowenfels, A.B., Bueno-de-Mesquita, H.B., Ghadirian, P., Baghurst, P.A., Zatonski, W.A., Miller, A.B., Duell, E.J., Boffetta, P. and Boyle, P., 2010. Past medical history and pancreatic cancer risk: Results from a multicenter case-control study. Ann Epidemiol 20, 92-98.

Maitra, A. and Hruban, R.H., 2008. Pancreatic cancer. Annu Rev Pathol 3, 157-188.

Mantell, D.J., Owens, P.E., Bundred, N.J., Mawer, E.B. and Canfield, A.E., 2000. 1α,25-dihydroxyvitamin D3 inhibits angiogenesis *in vitro* and *in vivo*. Circ Res 87, 214-220.

Mantovani, A., Allavena, P., Sica, A. and Balkwill, F., 2008. Cancer-related inflammation. Nature 454, 436-444.

Mathieu, C., Gysemans, C., Giulietti, A. and Bouillon, R., 2005. Vitamin D and diabetes. Diabetologia 48, 1247-57.

McCollum, E.V., Simmonds, N., Becker J.E. and Shipley, P.G., 1922. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J Biol Chem 53, 293-298.

McCollum, E.V., Simmonds,N. and Pitz,W., 1916. The realtion of the unidentified dietary factors, the fat-soluble A and watersoluble B of the iet to the growth promoting properties of milk. J Biol Chem 27, 33-38.

McGrath, J.L., Sukanta, S., Burne, T.H.J. and Eyles, D.W., 2010. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. J Steroid Biochem Mol Biol 121, 471-477.

Merke, J., Milde, P., Lewicka, S., Hügel, U., Klaus, G., Mangelsdorf, D.J., Haussler, M.R., Rauterberg, E.W. and Ritz, E., 1989. Identification and regulation of 1,25-dihydroxyvitamin D3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. J Clin Invest 83, 1903-1915.

Michaud, D.S., Wolpin, B., Giovannucci, E., Liu, S., Cochrane, B., Manson, J.E., Pollak, M.N., Ma, J. and Fuchs, C.S., 2007. Prediagnostic plasma C-peptide and pancreatic cancer risk in men and women. Cancer Epidemiol Biomarkers Prev 16, 2101-2109.

Michaud, D.S., Vrieling, A., Jiao, L., Mendelsohn, J.B., Steplowski, E., Lynch, S.M., Wactawski-Wende, J., Arslan, A.A., Bas Bueno-de-Mesquita, H., Fuchs, C.S., Gross, M., Helzlsouer, K., Jacobs, E.J., Lacroix, A., Petersen, G., Zheng, W., Allen, N., Ammundadottir, L., Bergmann, M.M., Boffetta, P., Buring, J.E., Canzian, F., Chanock, S.J, Clavel-Chapelon, F., Clipp, S., Freiberg, M.S., Michael Gaziano, J., Giovannucci, E.L., Hankinson, S., Hartge, P., Hoover, R.N., Allan, Hubbell. F., Hunter, D.J., Hutchinson, A., Jacobs, K., Kooperberg, C., Kraft, P., Manjer, J., Navarro, C., Peeters, P.H., Shu, X.O., Stevens, V., Thomas, G., Tjønneland, A., Tobias, G.S., Trichopoulos, D., Tumino, R., Vineis, P., Virtamo, J., Wallace, R., Wolpin, B.M., Yu, K., Zeleniuch-Jacquotte, A. and Stolzenberg-Solomon, R.Z., 2010. Alcohol intake and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium (PanScan). Cancer Causes Control 21, 1213-1225.

Miller, G.J., 1998. Vitamin D and prostate cancer: biologic interactions and clinical potentials. Cancer Metastasis Rev 17, 353-360.

Mohr, S.B., Garland, C.F., Gorham, E.D., Grant, W.B. and Garland, F.C., 2010. Ultraviolet B irradiance and vitamin D status are inversely associated with incidence rates of pancreatic cancer worldwide. Pancreas 39, 669-674.

Moore, M.J., Goldstein, D., Hamm, J., Figer, A., Hecht, J.R., Gallinger, S., Au, H.J., Murawa, P., Walde, D., Wolff, R.A., Campos, D., Lim, R., Ding, K., Clark, G., Voskoglou-Nomikos, T., Ptasynski, M., Parulekar, W. and the National Cancer Institute of Canada Clinical Trials Group, 2007. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25, 1960-1966.

Moreno, J., Krishnan, A.V. and Feldman, D., 2005. Molecular mechanisms mediating the anti-proliferative effects of vitamin D in prostate cancer. J Steroid Biochem Mol Biol 97, 31-36.

Mozolowski, W., 1939. On the cure of rickets. Nature 143, 121-124.

Nakagawa, K., Sasaki, Y., Kato, S., Kubodera, N. and Okano, T., 2005. 22-Oxa-1alpha,25-dihydroxyvitamin D3 inhibits metastasis and angiogenesis in lung cancer. Carcinogenesis 26, 1044-1054.

Neale, R.E., Youlden, D.R., Krnjacki, L., Kimlin, M.G. and Van der Pols, J.C., 2009. Latitude variation in pancreatic cancer mortality in Australia. Pancreas 38, 387-390.

Neoptolemos, J.P., Stocken, D.D., Friess, H., Bassi, C., Dunn, J.A., Hickey, H., Beger, H., Fernandez-Cruz, L., Dervenis, C., Lacaine, F., Falconi, M., Pederzoli, P., Pap, A., Spooner, D., Kerr, D.J., Büchler, M.W. and the European Study Group for Pancreatic Cancer, 2004. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 350, 1200-1210. Erratum in: N Engl J Med 351, 726.

Nitecki, S.S., Sarr, M.G., Colby, T.V. and Van Heerden, J.A., 1995. Long-term survival after resection for ductal adenocarcinoma of the pancreas. Is it really improving? Ann Surg 221, 59-66.

Nitsche, C., Simon, P., Weiss, F.U., Fluhr, G., Weber, E., Gärtner, S., Behn, C.O., Kraft, M., Ringel, J., Aghdassi, A., Mayerle, J. and Lerch, M.M., 2011. Environmental risk factors for chronic pancreatitis and pancreatic cancer. Dig Dis 29, 235-242.

Oberstein, P.E. and Wasif Saif, M., 2011. First-line treatment for advanced pancreatic cancer. Highlights from the 2011 ASCO Gastrointestinal Cancers Symposium. San Francisco, CA, USA. January 20-22, 2011.

Oettle, H., Post, S., Neuhaus, P., Gellert, K., Langrehr, J., Ridwelski, K., Schramm, H., Fahlke, J., Zuelke, C., Burkart, C., Gutberlet, K., Kettner, E., Schmalenberg, H., Weigang-Koehler, K., Bechstein., W., Niedergethmann, M., Schmidt-Wolf, I., Roll, L., Doerken, B. and Riess, H., 2007. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 297, 267-277.

Palm, T., 1890. The geographical distriubution and etiology of rickets. Practitiner 45, 270-279.

Palmer, H.G., 2003. Genetic signatures of differentiation induced by 1α,25-dihydroxyvitamin D3 in human colon cancer cells. Cancer Res 63, 7799-7806.

Pálmer, H.G., González-Sancho, J.M., Espada, J., Berciano, M.T., Puig, I., Baulida, J., Quintanilla, M., Cano, A., de Herreros, A.G., Lafarga, M. and Muñoz, A., 2001. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. Cell Biol 154, 369-387.

Patel, A.V., Rodriguez, C., Bernstein, L., Chao, A., Thun, M.J. and Calle, E.E., 2005. Obesity, recreational physical activity, and risk of pancreatic cancer in a large US Cohort. Cancer Epidemiol Biomarkers Prev 14, 459-466.

Peehl, D.M., Shinghal, R., Nonn, L., Seto, E., Krishnan, A.V., Brooks, J.D. and Feldman, D., 2004. Molecular activity of 1,25-dihydroxyvitamin D3 in primary cultures of human prostatic epithelial cells revealed by cDNA microarray analysis. J Steroid Biochem Mol Biol 92, 131-141.

Pendás-Franco, N., García, J.M., Peña, C., Valle, N., Pálmer, H.G., Heinäniemi, M., Carlberg, C., Jiménez, B., Bonilla, F., Muñoz, A. and González-Sancho, J.M., 2008. DICKKOPF-4 is induced by TCF/beta-catenin and upregulated in human colon cancer, promotes tumour cell invasion and angiogenesis and is repressed by 1alpha,25-dihydroxyvitamin D3. Oncogene 27, 4467-4477.

Persons, K.S., Eddy, V.J., Chadid, S., Deoliveira, R., Saha, A.K. and Ray, R., 2010. Anti-growth effect of 1,25-dihydroxyvitamin D3-3-bromoacetate alone or in combination with 5-amino-imidazole-4-carboxamide-1-beta-4-ribofuranoside in pancreatic cancer cells. Anticancer Res 30, 1875-1880.

Pettersson, F., Colston, K.W. and Dalgleish, A.G., 2000. Differential and antagonistic effects of 9-cis-retinoic acid and vitamin D analogues on pancreatic cancer cells *in vitro*. Br J Cancer 83, 239-245.

Plum, L.A. and DeLuca, H.F., 2010. Vitamin D, disease and therapeutic opportunities. Nature Rev (Drug Discovery) 9, 941-955.

Ponchon, G. and DeLuca, H.F., 1969. The role of the liver in the metabolism of vitamin D. J Clin Invest 48, 1273-1279.

Powers, J.T., Hong, S., Mayhew, C.N., Rogers, P.M., Knudsen, E.S. and Johnson, D.G., 2004. E2F1 uses the ATM signaling pathway to induce p53 and Chk2 phosphorylation and apoptosis. Mol Cancer Res 2, 203-214.

Raimondi, S., Maisonneuve, P. and Lowenfels, A.B., 2009. Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol 6, 699-708.

Ramagopalan, S. V., Heger, A., Berlanga, A. J., Maugeri, N. J., Lincoln, M. R., Burrell, A., Handunnetthi, L., Handel, A.E., Disanto, G., Orton, S.M., Watson, C.T., Morahan, J.M., Giovannoni, G., Ponting, C.P., Ebers, G.C. and Knight, J.C., 2010. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 20, 1352-1360.

Redston, M.S., Caldas, C., Seymour, A.B., Hruban, R.H., da Costa, L., Yeo, C.J. and Kern, S.E., 1994. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. Cancer Res 54, 3025-3033.

Reeves, G.K., Pirie, K., Beral, V., Green, J., Spencer, E. and Bull, D., 2007. Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. BMJ 335, 1134.

Regine, W.F., Winter, K.A., Abrams, R.A., Safran, H., Hoffman, J.P., Konski, A., Benson, A.B., Macdonald, J.S., Kudrimoti, M.R., Fromm, M.L., Haddock, M.G., Schaefer, P., Willett, C.G. and Rich, T.A., 2008. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. JAMA 299, 1019-1026.

Renouf, D. and Moore, M., 2010. Evolution of systemic therapy for advanced pancreatic cancer. Expert. Rev. Anticancer Ther 10, 529-540.

Resendes, A.R., Majó, N., Segalés, J., Espadamala, J., Mateu, E., Chianini, F., Nofrarías, M.and Domingo, M., 2004. Apoptosis in normal lymphoid organs from healthy normal, conventional pigs at different ages detected by TUNEL and cleaved caspase-3 immunohistochemistry in paraffin-embedded tissues. Vet Immunol Immunopathol 99, 203-213.

Rohrmann, S., Linseisen, J., Vrieling, A., Boffetta, P., Stolzenberg-Solomon, R.Z., Lowenfels, A.B., Jensen, M.K., Overvad, K., Olsen, A., Tjonneland, A., Boutron-Ruault, M.C., Clavel-Chapelon, F., Fagherazzi, G., Misirli, G., Lagiou, P., Trichopoulou, A., Kaaks, R., Bergmann, M.M., Boeing, H., Bingham, S., Khaw, K.T., Allen, N., Roddam, A., Palli, D., Pala, V., Panico, S., Tumino, R., Vineis, P., Peeters, P.H., Hjartåker, A., Lund, E., Redondo Cornejo, M.L., Agudo, A., Arriola, L., Sánchez, M.J., Tormo, M.J., Barricarte Gurrea, A., Lindkvist, B., Manjer, J., Johansson, I., Ye, W., Slimani, N., Duell, E.J., Jenab, M., Michaud, D.S., Mouw, T., Riboli, E. and Bueno-de-Mesquita, H.B., 2009. Ethanol intake and the risk of pancreatic cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Causes Control 20, 785-794.

Santibanez, M., Vioque, J., Alguacil, J., Garcia de la Hera, M., Moreno-Osset, E., Carrato, A., Porta, M. and Kauppinen, T., 2010. Occupational exposures and risk of pancreatic cancer. Eur J Epidemiol 25, 721-730.

Sato, N., Maitra, A., Fukushima, N., van Heek, N.T., Matsubayashi, H., Iacobuzio-Donahue, C.A., Rosty, C., Goggins, M., 2003. Frequent hypomethylation of multiple genes overexpressed in pancreatic ductal adenocarcinoma. Cancer Res 63, 4158-4166.

Schatzkin, A. and Stolzenberg-Solomon, R.Z., 2009. Alcohol use and risk of pancreatic cancer: the NIH-AARP Diet and Health Study. Am J Epidemiol 169, 1043-1051.

Schuster, I., 2011. Cytochromes P450 are essential players in the vitamin D signaling system. Biochim Biophys Acta 1814, 186-199.

Schwartz, G.G. and Hulka, B.S., 1990. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Res 10, 1307-1311.

Schwartz, G.G., Eads, D., Rao, A., Cramer, S.D., Willingham, M.C., Chen, T.C., Jamieson, D.P., Wang, L., Burnstein, K.L., Holick, M.F. and Koumenis, C., 2004. Pancreatic cancer cells express 25-hydroxyvitamin D-1 alpha-hydroxylase and their proliferation is inhibited by the prohormone 25-hydroxyvitamin D3. Carcinogenesis 25, 1015-1026.

Schwartz, G.G., Eads, D., Naczki, C., Northrup, S., Chen, T. and Koumenis, C., 2008. 19-nor-1 alpha,25-dihydroxyvitamin D2 (paricalcitol) inhibits the proliferation of human pancreatic cancer cells *in vitro* and *in vivo*. Cancer Biol Ther 7, 430-436.

Siegel, R., Naishadham, D. and Jemal, A. Cancer statistics, 2012. CA Cancer J Clin 62, 10-29.

Signorello, L.B., Shi, J., Cai, Q., Zheng, W., Williams, S.M., Long, J., Cohen, S.S., Li, G., Hollis, B.W., Smith, J.R. and Blot, W.J., 2011. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D Levels among African Americans. PLoS One 6, e28623.

Skinner, H.G., Michaud, D.S., Giovannucci, E., Willett, W.C., Colditz, G.A. and Fuchs, C.S., 2006. Vitamin D intake and the risk for pancreatic cancer in two cohort studies. Cancer Epidemiol Biomarkers Prev 15, 1688-1695.

Skowronski, R.J., Peehl, D.M. and Feldman, D., 1993. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines. Endocrinology 132, 1952-1960.

Steenbock, H. and Black, A., 1924. Fat-soluble vitamins XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultraviolet light. J Biol Chem 61, 405-422.

Stolzenberg-Solomon, R.Z., Graubard, B.I., Chari, S., Limburg, P., Taylor, P.R., Virtamo, J. and Albanes, D., 2005. Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. JAMA 294, 2872-2878.

Stolzenberg-Solomon, R.Z., Hayes, R.B., Horst, R.L., Anderson, K.E., Hollis, B.W. and Silverman, D.T., 2009. Serum vitamin D and risk of pancreatic cancer in the prostate, lung, colorectal, and ovarian screening trial. Cancer Res 69, 1439-1447.

Stolzenberg-Solomon, R.Z., Jacobs, E.J., Arslan, A.A.; Qi, D., Patel, A.V., Helzlsouer, K.J., Weinstein, S.J., McCullough, M.L., Purdue, M.P., Shu, X.O., Snyder, K., Virtamo, J., Wilkins, L.R., Yu, K., Zeleniuch-Jacquotte, A., Zheng, W., Albanes, D., Cai, Q., Harvey, C., Hayes, R., Clipp, S., Horst, R.L., Irish, L., Koenig, K., Le Marchand, L. and Kolonel, L.N., 2010. Circulating 25-hydroxyvitamin D and risk of pancreatic cancer: cohort consortium vitamin D pooling project of rarer cancers. Am J Epidemiol 172, 81-93.

Stolzenberg-Solomon, R.Z., Vieth, R., Azad, A., Pietinen, P., Taylor, P.R., Virtamo, J. and Albanes D., 2006. A prospective nested case-control study of vitamin D status and pancreatic cancer risk in male smokers. Cancer Res 66, 10213-10219.

Stumpf, W.E., Sar, M., Reid, F.A., Tanaka, Y. and DeLuca, H.F., 1979. Target cells for 1,25-dihydroxyvitamin D3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. Science 206, 1188-1190.

Takaori, K., 2007. Current understanding of precursors to pancreatic cancer. J. Hepatobiliary Pancreat Surg 14, 217-223.

Talamini, R., Polesel, J., Gallus, S., Dal Maso, L., Zucchetto, A., Negri, E., Bosetti, C., Lucenteforte, E., Boz, G., Franceschi, S., Serraino, D. and La Vecchia, C., 2010. Tobacco smoking, alcohol consumption and pancreatic cancer risk: A case-control study in Italy. Eur J Cancer 46, 370-376.

Tong, W.M., Hofer, H., Ellinger, A., Peterlik, M. and Cross, H.S., 1999. Mechanism of antimitogenic action of vitamin D in human colon carcinoma cells: relevance for suppression of epidermal growth factor-stimulated cell growth. Oncol Res 11, 77-84.

Tramacere, I., Scotti, L., Jenab, M., Bagnardi, V., Bellocco, R., Rota, M., Corrao, G., Bravi, F., Boffetta, P. and La Vecchia, C., 2010 Alcohol drinking and pancreatic cancer risk: a meta-analysis of the dose-risk relation. Int J Cancer 126, 1474-1486.

Trede, M., Schwall, G. and Saeger, H.D., 1990. Survival after pancreatoduodenectmy. 118 Consecutive resections without an operative mortality. Ann Surg 211, 447-458.

Tuohimaa, P., Pukkala, E., Scelo, G., Olsen, J.H., Brewster, D.H., Hemminki, K., Tracey, E., Weiderpass, E., Kliewer, E.V., Pompe-Kirn, V., McBride, M.L., Martos, C., Chia, K.S., Tonita, J.M., Jonasson, J.G., Boffetta, P.and Brennan, P., 2007. Does solar exposure, as indicated by the non-melanoma skin cancers, protect from solid cancers: vitamin D as a possible explanation. Eur J Cancer 43, 1701-1712.

Vogelstein, B. and Kinzler, K.W., 2004. Cancer genes and the pathways they control. Nat Med 10, 789-799.

Vrieling, A., Verhage, B.A., Van Duijnhoven, F.J., Jenab, M., Overvad, K., Tjonneland, A., John, T., Dimosthenes, Z., Pall,i D., Sieri, S., Mattiello, A., Tumino, R., Vineis, P., van Gils, C.H., Peeters, P.H., Engeset, D., Lund, E., Rodríguez, Suárez. L., Jakszyn, P., Larrañaga, N., Sánchez, M.J., Chirlaque, M.D., Ardanaz, E., Manjer, J., Lindkvist, B., Hallmans, G., Ye, W., Bingham, S., Khaw, K.T., Roddam, A., Key, T., Boffetta, P., Duell, E.J., Michaud, D.S., Riboli, E. and Bueno-de-Mesquita, H.B., 2009. Fruit and vegetable consumption and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer 124, 1926-1934.

Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., Van Meurs, J.B., Berry, D., Kiel, D.P., Streeten, E.A., Ohlsson, C., Koller, D.L., Peltonen, L., Cooper, J.D., O'Reilly, P.F., Houston, D.K., Glazer, N.L., Vandenput, L., Peacock, M., Shi, J., Rivadeneira, F., McCarthy, M.I., Anneli, P., De Boer, I.H., Mangino, M., Kato, B., Smyth, D.J., Booth, S.L., Jacques, P.F., Burke, G.L., Goodarzi, M., Cheung, C.L., Wolf, M., Rice, K., Goltzman, D., Hidiroglou, N., Ladouceur, M., Wareham, N.J., Hocking, L.J., Hart, D., Arden, N.K., Cooper, C., Malik, S., Fraser, W.D., Hartikainen, A.L., Zhai, G., Macdonald, H.M., Forouhi, N.G., Loos, R.J., Reid, D.M., Hakim, A., Dennison,

E., Liu, Y., Power, C., Stevens, H.E., Jaana, L., Vasan, R.S., Soranzo, N., Bojunga, J., Psaty, B.M., Lorentzon, M., Foroud, T., Harris, T.B., Hofman, A., Jansson, J.O., Cauley, J.A., Uitterlinden, A.G., Gibson, Q., Järvelin, M.R., Karasik, D., Siscovick, D.S., Econs, M.J., Kritchevsky, S.B., Florez, J.C., Todd, J.A., Dupuis, J., Hyppönen, E. and Spector, T.D., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180-188.

Welsh, J., 2011. Cellular and molecular effects of vitamin D on carcinogenesis. Arch Biochem Biophys 523, 107-114.

Wolpin, B.M., Ng, K., Bao, Y., Kraft, P., Stampfer, M.J., Michaud, D.S., Ma, J., Buring, J.E., Sesso, H.D., Lee, I.M., Rifai, N., Cochrane, B.B., Wactawski-Wende, J., Chlebowski, R.T., Willett, W.C., Manson, J.E., Giovannucci, E.L. and Fuchs, C.S., 2012. Plasma 25-hydroxyvitamin d and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev 21, 82-91.

Yachida, S. and Iacobuzio-Donahue, C.A., 2009. The pathology and genetics of metastatic pancreatic cancer. Arch Pathol Lab Med 133, 413-422.

Yanagisawa, J., Yanagi, Y., Masuhiro, Y., Suzawa, M., Watanabe, M., Kashiwagi, K., Toriyabe, T., Kawabata., M., Miyazono, K. and Kato, S., 1999. Convergence of transforming growth factor-β and vitamin D signalling pathways on SMAD transcriptional coactivators. Science 283, 1317-1321.

Yang, E.S. and Burnstein, K.L., 2003. Vitamin D inhibits G1 to S progression in LNCaP prostate cancer cells through p27Kip1 stabilization and Cdk2 mislocalization to the cytoplasm. J Biol Chem 278, 46862-46868.

Yang, E.S., Maiorino, C.A., Roos, B.A., Knight, S.R. and Burnstein, K.L., 2002. Vitamin D-mediated growth inhibition of an androgen-ablated LNCaP cell line model of human prostate cancer. Mol Cell Endocrinol 186, 69-79.

Yeo, C.J., Cameron, J.L., Sohn, T.A., Lillemoe, K.D., Pitt, H.A., Talamin,i M.A., Hruban, R.H., Ord, S.E., Sauter, P.K., Coleman, J., Zahurak, M.L., Grochow, L.B. and Abrams, R.A., 1997. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: pathology, complications, and outcomes. Ann Surg 226, 248-257; discussion 257-260.

Yin, L., Grandi, N., Raum, E., Haug, U., Arndt, V. and Brenner, H., 2010. Meta-analysis: serum vitamin D and breast cancer risk. Eur J Cancer 46, 2196-2205.

Ylikomi, T., Laaksi, I., Lou, Y.R., Martikainen, P., Miettinen, S., Pennanen, P., Purmonen, S., Syvala, H., Vienonen, A. and Tuohimaa, P., 2002. Antiproliferative action of vitamin D. Vitam Horm 64, 357-406.

Yonezawa, S., Higashi, M., Yamada, N. and Goto, M., 2008 Precursor lesions of pancreatic cancer. Gut Liver 2, 137-154.

Yu, J., Li, A., Hong, S.M., Hruban, R.H. and Goggins, M., 2011. MicroRNA Alterations of Pancreatic Intraepithelial Neoplasms (PanINs). Clin Cancer Res 18 981-992.

Zehnder, D., Bland, R., Williams, M.C., McNinch, R.W., Howie, A.J., Stewart, P.M. and Hewison, M., 2001. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 86, 888-894.

Zinser, G.M., McEleney, K. and Welsh, J.E., 2003. Characterization of mammary tumor cell lines from wild type and vitamin D receptor knockout mice. Mol. Cell Endocrinol 200, 67-80.

Zugmaier, G., Jäger, R., Grage, B., Gottardis, M.M., Havemann, K. and Knabbe, C., 1996. Growth-inhibitory effects of vitamin D analogues and retinoids on human pancreatic cancer cells. Br J Cancer 73, 1341-1346.

Key facts

- Melanoma is the most commonly lethal form of skin cancer, and although it is known to be caused by sun exposure, the relationship between patterns of sun exposure and risk is very complex.
- Sun exposure is critical to human health, however, in terms of synthesis of vitamin D, low vitamin D is associated with increased risk of many diseases.
- The balance between sun exposure and protection is therefore the key issue for melanoma and that will depend on where people live, their skin type (how sensitive they are to sunburn), their lifestyle (especially behaviour in the sun) and cultural clothing requirements.
- Although epidemiological studies consistently implicate sunburn as a major risk factor for melanoma, there is some evidence that regular moderate sun exposure might be protective for melanoma.
- Vitamin D levels may also be important to survival for melanoma patients but we don't yet know the optimal blood levels.

Summary points

- Excessive sun exposure increases the risk of melanoma of the skin in pale skinned peoples.
- The role of vitamin D in protection from melanoma is not known. There is some evidence that pale-skinned people who spend a lot of time in the sun in temperate climates may be at a reduced risk compared with those who spend most of their time indoors.
- There appear to be differences in the relationship between sustained sun exposure and melanoma risk at different latitudes for pale-skinned people.
- There are some genetic data looking at inheritance of the gene coding for the vitamin D receptor, for a role for vitamin D in the cause of melanoma.
- Melanoma patients in the Leeds Melanoma Cohort who have lower levels of vitamin D at diagnosis have thicker, poorer prognosis tumours and are more likely to die of their melanoma.
- Blood levels of vitamin D are affected by obesity, sun exposure, dietary supplementation and genetics. Variation in the gene coding for vitamin D binding protein plays a significant role in determining blood levels.
- Vitamin D has an anti-proliferative effect on melanoma cells *in vitro* and therefore higher levels may be beneficial in terms of survival for melanoma patients, however we have concerns to avoid excessively high levels of vitamin D fearing an adverse effect on immune-surveillance at high doses. A conservative level to aim for might be 60 to 85 nmol/l throughout the year.

22. Vitamin D and melanoma

J.A. Newton-Bishop and J.A. Randerson-Moor
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds LS9 7TF, United Kingdom; j.a.newton-bishop@leeds.ac.uk

Abstract

Melanoma is predominantly a cancer of pale-skinned peoples and there is no doubt that sunburn is the best short-term measure known of the adverse effects of intense UV exposure. The genetics of susceptibility reinforce the role of sunburn as the commonest susceptibility genes are associated with sun-sensitivity (pigment genes). Other common genes are also naevus genes and there are newly identified susceptibility genes of unknown role which when further studied will tell us more about the biological basis of susceptibility. While observational studies, proving a role for vitamin D in susceptibility and survival from melanoma, are not achievable, there are some lines of circumstantial evidence, which can be interpreted this way. A case-control study suggests that moderate regular sun exposure may be protective for melanoma and this could be explained by photoadaption after sun exposure having the effect of reducing the likelihood of sunburn, or a protective role for vitamin D. Genetic association studies of inheritance of polymorphisms in the gene encoding the vitamin D receptor are suggestive of a role for vitamin D in protection from melanoma but this is as yet unproven. Melanoma patients in the Leeds Melanoma Cohort who have lower levels of vitamin D at diagnosis have thicker tumours and poorer prognosis even when the data are stratified for the thickness of the tumours, but these data have not as yet been confirmed in other studies. There are *in vitro* data to suggest that vitamin D is anti-proliferative for melanoma cells and the putative effect of vitamin D on melanoma survival might be attributed to those anti-proliferative effects. Vitamin D however has complex effects on the immune system, some of which might be beneficial for melanoma patients and some theoretically harmful. Consequently the optimal approach for managing vitamin D levels after diagnosis of melanoma (especially when melanoma patients tend to be sun-avoidant after diagnosis) is not known. A sensible approach might be to aim for levels at the lower end of the normal range (e.g. 60 to 85 nmol/l), whilst this difficult issue is being resolved.

Keywords: optimal levels, prevention, survival, immune-surveillance, sunburn

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_22, © Wageningen Academic Publishers 2013

Abbreviations

CI	Confidence interval
CRP	C-reactive protein
MC1R	Melanocortin 1 receptor
OR	Odds ratio
SNP	Single nucleotide polymorphism
UVB	Ultraviolet B
VDR	Vitamin D receptor
Vitamin D	25-hydroxyvitamin D3

22.1 Introduction

Melanoma of the skin is the most potentially lethal form of skin cancer. It arises either from a benign melanocytic naevus or *de novo*. Most cases arise on sun-exposed skin in pale skinned peoples, although rare subtypes occur on sun-protected body sites at approximately the same incidence in all ethnic groups. The commonest type is the superficial spreading type, which is most common on the back of men and the legs of women. The nodular subtype is a less common, more rapidly growing sub-type and finally, melanomas also arise from precursor lesions called lentigo maligna, on the chronically sun-exposed skin of the face. The incidence of melanoma on sun-exposed skin has increased considerably in most white skinned populations and this is attributed to greater exposure to sunny holidays or leisure time sun exposure. Although most melanomas are cured by excision, the treatment of metastatic melanoma remains very difficult because of resistance to chemotherapy.

22.2 Melanoma susceptibility genes

The majority of cases of melanoma are of the superficial spreading or nodular varieties and these occur rarely in individuals who do not have white skin, so genes are clearly important in susceptibility. Within the white-skinned populations, those with pale skin which has a tendency to burn are especially at risk (Gandini *et al.*, 2005c). A tendency to burn in the sun is related to inherited pigmentary genotypes such as variants in the gene coding for the MC1R and pigment genes, such as *MC1R*, are also known to be associated with melanoma risk (Valverde *et al.*, 1996). Genome wide association studies have recently identified SNPs in a number of additional pigment genes such as tyrosinase and the agouti locus (an MC1R agonist) (Gudbjartsson *et al.*, 2008) as melanoma risk alleles. Thus melanoma is actually a strongly genetically determined type of cancer and a number of the susceptibility genes are pigment genes related to how skin reacts to sunlight.

There are other groups of susceptibility genes, which are not pigment genes. The strongest phenotypic risk factor for melanoma is the presence of multiple melanocytic naevi (moles) (Gandini *et al.*, 2005a). Individuals with multiple moles also commonly have clinically atypical

moles, which are larger than the norm (>5 mm in diameter), with an irregular shape and colour. This phenotype is commonly known as the atypical mole syndrome phenotype. Twin studies showed that this phenotype is predominantly genetically determined (Easton *et al.*, 1991; Wachsmuth *et al.*, 2001) and therefore, not surprisingly, the second group of identified susceptibility genes are genes also associated with naevus number, such as a locus on chromosome 9, *PLA2G6* (Falchi *et al.*, 2009) and a gene called *IRF4* (Duffy *et al.*, 2010). Although this 'at risk' phenotype is primarily genetically determined, there is a measurable effect of sun exposure seen both in twin studies (Wachsmuth *et al.*, 2005) and in melanoma case-control studies such as one recently reported by our group in which increased number of naevi was related especially to sunny holidays (Newton-Bishop *et al.*, 2010). Some have considered the development of melanoma in people with many naevi as occurring by a different 'route' than those developing as a result of inherited pigment genes (Whiteman *et al.*, 2003), but it can be seen that sun exposure is likely critical for both.

Very recent genome wide association studies have identified new groups of susceptibility genes, in the vicinity of genes such as *MX2* (Barrett *et al.*, 2011) which have not previously been associated with melanoma biology and are not related to the known melanoma-associated risk factors of pigmentation and naevus count, and hence likely indicate different pathogenetic 'routes' to melanoma, and exploration of these genes is likely to cast new light on pathogenesis in the near future.

In terms of genetic predisposition to melanoma then, pigment genes are important as are genes associated with increased numbers of naevi, and there are emerging data suggestive of genes not associated either with fair skin or increased numbers of naevi, and these remain to be understood.

22.3 Sun exposure and risk

That genes associated with a tendency to burn in the sun are melanoma susceptibility genes, and that the 'at risk' naevus phenotype is related to sunny holidays is good evidence for a role for sun exposure in the cause of melanoma. Epidemiological evidence is also strong. The world variation in occurrence shows that melanoma incidence is highest where pale-skinned people live closest to the equator. The highest incidence is in Australasia where the melanoma population is primarily genetically derived from the UK (Bishop *et al.*, 2009) and is therefore pale-skinned. The high incidence in Australasia has resulted from a combination of inherited fair skin genes and very high levels of sun exposure, for a population, which, it might be said, had evolved to thrive in northern Europe.

Case-control studies have provided very strong evidence for a role for sunburn in the pathogenesis of melanoma, reported as a meta-analysis (Gandini *et al.*, 2005b) and more recently as a pooled data analysis (Chang *et al.*, 2009) of multiple such studies. In both a very consistently elevated risk associated with sunburn was seen for melanoma at all latitudes. Many studies have reported a particularly strong effect for sunburn in childhood. These epidemiological data identifying sunburn as a risk factor are consistent with laboratory data developed in a fish model in which

intense ultraviolet B exposure in adults was associated with the development of melanoma (Fernandez *et al.*, 2012). The relative importance of early sunburn is supported by data summarized by Noonan (Noonan *et al.*, 2003) in which early exposure to ultraviolet light was necessary to initiate melanoma in hepatocytes growth factor/ scatter factor transgenic mice (Zaidi *et al.*, 2011).

Although there is no doubt that sunburn is associated with melanoma risk, there is a much less clear relationship between chronic sun exposure and risk. There is (at least in temperate climates) no dose response relationship between cumulative sun exposure and risk (Gandini *et al.*, 2005b) so that there appears to be something particular about sunburn which causes melanoma even if the total cumulative sun exposure through life is not high.

The mechanisms via which intense sun exposure, and consequent sunburn, increase melanoma risk were first extensively explored by Margaret Kripke and her colleagues. Using experiments in mice, they showed that ultraviolet B exposure may result both in local immunosuppression in the skin and systemic immunosuppression, and that these effects are related to growth of melanomas in the skin of those mice (Donawho and Kripke, 1991; Fisher and Kripke, 1977; 2002). The cellular and molecular basis of this UV-induced immunosuppression in man is now fairly well understood. The chromophores which mediate UV-induced immunosuppression in animals, including man, include products of damaged DNA (pyrimidine dimers), urocanic acid and membrane components which generate free radicals and oxidative stress (see review by Norval, 2011). UV-induced changes in the chromophores result in the release of inflammatory mediators, and migration of Langerhans and dermal dendritic cells from the skin to the draining lymph nodes. Immunosuppression involves production of T regulatory cells, which secrete the immunosuppressive cytokine IL-10. The accumulation of macrophages and mast cells in the skin also occurs. The theory developed by Kripke and her colleagues and reviewed eloquently by Norval (McGill *et al.*, 2002; Norval, 2011) is that UV exposure, if intense, may induce genetic mutations in the skin as well as local and systemic immunosuppression so that those mutant cells can proliferate unchecked. In this way they have proposed an explanation for the peculiar relationship between sunburn and melanoma risk. The propensity to melanoma as a result of sunburn is postulated also to result from the fact that melanocytes are highly differentiated cells, which are protected from apoptosis due to high expression of molecular inhibitors of apoptosis such as Bcl-2 (McGill *et al.*, 2002). It is postulated therefore, that mutated melanocytes are likely to persist in sunburn damaged cells because the mutated cells are less likely to apoptose than keratinocytes and less likely to be killed by immune-defence systems.

There is therefore no doubt that sun exposure inducing sunburn is strongly related to melanoma risk, and the concern is that the incidence of melanoma continues to rise in many countries predominantly populated by pale-skinned peoples who have access to sunny holidays (Downing *et al.*, 2006), given that holiday sun exposure is the behaviour most strongly related to sunburn in epidemiological studies (Newton-Bishop *et al.*, 2011). The difficulty however in converting the epidemiological data to public health advice, is the lack of a simple relationship between cumulative sun exposure and risk. If melanoma aetiology was related to sun exposure (some have argued) then there would be a linear relationship between cumulative sun exposure and

risk as there is, for example, for squamous cell carcinoma. There is in fact, for melanoma, little epidemiological evidence of such a relationship except in countries near to the equator such as Australia and Hawaii (Chang *et al.*, 2009). In temperate climates there are data which suggest the reverse, in that outdoor workers in some case-control studies appeared to be at somewhat reduced risk (Gandini *et al.*, 2005b). Sceptics have therefore doubted the relationship between sun exposure and risk.

We have recently reported a very detailed exploration of different patterns of sun exposure and melanoma risk in the UK (Newton-Bishop *et al.*, 2011) in 960 melanoma cases and 500 controls. In this study the clear increased risk associated with sunburn was seen, but 5 or more hours of regular sun exposure per day at weekends was actually associated with a reduced melanoma risk (OR 0.67, 95% CI 0.50-0.89 for highest versus lowest tertile of weekend exposure). This pattern of sun exposure was associated with higher blood levels of 25-hydroxyvitamin D3 (henceforth referred to as vitamin D when referring to serum levels) and this might suggest that vitamin D was protective for melanoma. Certainly vitamin D appears to be important in the skin's responses to the sun (see below) but there are a number of additional explanations for this finding. Regular weekend sun exposure is likely to result in 'photoadaption'; a process by which regular sun exposure results in a number of changes in the skin including tanning and epidermal thickening, which make sunburn less likely on sunny days. Thus this type of exposure might, in the long term, be protective for melanoma by reducing sunburn; although there are data to suggest that UV-induced immunosuppression will not necessarily be reduced as a result of photoadaption (Norval and Wulf, 2009). There may be alternative explanations too, such that higher vitamin D levels might be a marker of another (as yet unidentified) protective factor. However, vitamin D does remain a possibility in terms of melanoma prevention (not least because it is produced within the skin) and thus we will explore the significance of the published data.

There are very few data on vitamin D intake and risk. One small study from Italy showed some evidence of risk associated with lower reported levels of intake of vitamin D (Vinceti *et al.*, 2011), the adverse effect of low intake on risk being stronger in males.

In the Leeds case-control study there was some suggestion of a case-control difference in vitamin D levels in that cases had non-statistically significant lower levels than controls (Newton-Bishop *et al.*, 2011), but such studies are subject to considerable difficulties in interpretation because of effects consequent upon the diagnosis and case-control bias. Cohort studies of healthy individuals and risk are therefore much more likely to yield meaningful results. In the Women's Health Initiative Randomized Controlled Trial 36,282 post menopausal women were randomized to receive calcium and 400 IU vitamin D3 per day or placebo, with a mean follow up of 7 years (Tang *et al.*, 2011). Although there was no effect overall of vitamin D3 and melanoma risk, there was a reduction in risk for women who had previously had non-melanoma skin cancer (hazard ratio 0.43, 95% CI 0.21-0.90). Moreover, examination of the published Kaplan-Meier estimates of the cumulative hazard ratio for melanoma is suggestive of the emergence of reduced melanoma risk just beginning, after what is actually a small period of time (7 years) for a cancer which is thought

to result at least in part from sunburn in childhood. We could interpret this study as giving some support for the view that vitamin D might reduce melanoma risk, but take a cautious view as yet.

22.4 Vitamin D and the skin

Vitamin D synthesis starts in the skin after irradiation with UVB, when UVB-mediated photolysis of 7-dehydrocholesterol occurs to form pre-vitamin D3. Subsequent enzymic conversion occurs outside the skin predominantly, although both the necessary enzymes, 25-hydroxylase (CYP27A1) and 1α-hydroxylase (CYP27B1) are present in skin, in keratinocytes. Thus the active form of vitamin D, 1,25-dihydroxyvitamin D3 may be present in the skin and what is known about its function there suggests that it might be photoprotective as it enhances repair of pyridimine dimers and, in some circumstances, protects against UV-induced immunomodulation. It is clear that injury to the skin moreover results in increased synthesis of vitamin D in the skin and there is evidence that the vitamin D then increases the expression of pattern recognition receptors important in innate immunity, such as toll-like receptors and CD14 (Miller and Gallo, 2010). Innate immunity is classically described as important in early defence systems. So, cutaneous vitamin D might protect against malignancy by increasing DNA repair or via a role in innate immunity. Certainly vitamin D deficiency is thought to be related to an increased tendency to infection, as was seen in terms of chest infections in children with rickets in the past. Dermatologists are also familiar with the use of ultraviolet light to treat cutaneous tuberculosis (Norval, 2011) but it's not clear whether the beneficial effects could be attributed to the UV exposure itself or consequent vitamin D synthesis.

Although cutaneous vitamin D may have a protective function by playing a role in innate immunity, it is known to suppress Th1 and Th17 cytokine responses thus potentially leading to suppression of adaptive immune responses (Norval, 2011), and there is clinical evidence for suppression of immunity as sun exposure commonly results in reactivation of latent viral infections with herpes simplex. Exposure to intense ultraviolet light can moreover also suppress immunity both in the skin and systematically as described above, so that the net effect of sun exposure on cutaneous immunity is likely to depend upon many factors such as UV dosage and possibly the genetic characteristics of the host.

In summary then, it is possible that our observation that regular weekend sun exposure in the summer in the UK was protective for melanoma and associated with higher serum vitamin D levels whilst sunburn was associated with risk, might reflect this complex interaction between sun exposure, vitamin D synthesis and immunity. One interpretation might be that moderate but persistent sun exposure results in increased levels of cutaneous vitamin D, thereby controlling innate immunity in the skin and providing protection from the profound negative effects on the immune system resulting from sudden intense sun exposure as suggested by Dixon *et al* (2010). The ability of an individual to respond to regular sun exposure in terms of protection from melanoma would however depend upon the individual's genetically determined ability to

synthesise vitamin D in the skin, their pigmentation, genetic variation in DNA repair capabilities, or innate immunity, use of clothing, etc.

22.5 Genetic data

Sun exposure, if intense and especially if associated with sunburn, is clearly causal for melanoma. However if there is a complex relationship between doses of sun exposure and risk, so that moderate but sustained exposures might protect against melanoma by resulting in higher levels of vitamin D, then elucidating the relationships involved in the effects of sunshine on the skin is understandably difficult. A number of groups have therefore sought to use genetic association studies to study this relationship. The genomic effects of vitamin D on the cell are mediated by binding of vitamin D to a heterodimer made up of the nuclear VDR and the retinoid receptor, RXR. The binding results in transcription of a large number of genes explored recently using ChIP sequencing (Ramagopalan *et al.*, 2010) in which 229 genes were identified with significant changes in response to vitamin D. It is thought that vitamin D may also have transcription-independent effects mediated by binding elsewhere possibly even to cell surface molecules such as the epidermal growth factor, summarized by Deeb *et al* (2007), but these effects are less well studied. The changes consequent upon these non-genomic effects of vitamin D however are much more rapid then the genomic effects (Dixon *et al.*, 2010) taking seconds or minutes rather than hours or days.

The gene coding for the VDR is polymorphic and a number of SNPs in the gene are thought to modify the expression and/or activity of the receptor. The functional implications of six variants in particular have been extensively investigated: the coding non-synonymous variant FokI (p.Met1Thr, rs2228570); the promoter SNPs Cdx-2 (rs11568820) and GATA (A-1012G, rs4516035); and the 3' gene SNPs BsmI (rs1544410), ApaI (rs7975232) and TaqI (rs731236). The FokI 'T' variant located in exon 2 of the *VDR* gene, the only non-synonymous *VDR* SNP so far identified, leads to the creation of an alternative start site and results in the expression of an extended VDR protein (427aa) which has been shown to both have diminished transcriptional activity compared to the shorter 'C' variant protein (424aa) (Colin *et al.*, 2000; Jurutka *et al.*, 2000; Whitfield *et al.*, 2001) as well as less efficient interaction with the TFIIB transcription factor (Jurutka *et al.*, 2000) suggesting that the 'C' variant protein represents a more transcriptionally potent VDR protein. Additionally two promoter variants which affect a Cdx-2 and GATA-3 transcription binding site respectively have also been shown to alter *VDR* expression. The Cdx-2 'A' variant has been shown to bind the intestinal specific transcription factor Cdx-2 more strongly than the wild type 'G' variant with a concurrent increase in transcriptional activity (Arai *et al.*, 2001). It is proposed that the 'A' allele leads to increased intestinal VDR expression and thus better absorption of calcium (reviewed by Uitterlinden *et al.*, 2004). The GATA 'G' variant results in destruction of a strong GATA-3 core binding site (Halsall *et al.*, 2004). The GATA-3 transcription factor is reported to be important in directing the polarization of naïve T-cells to Th2 (T-helper Type 2) lymphocytes (Rengarajan *et al.*, 2000) and has been shown to be upregulated by $1,25(OH)_2D3$ (Boonstra *et al.*, 2001). Consequently the *VDR* GATA polymorphism is believed to

have a role in the anticancer immune response (Halsall *et al.*, 2004). The functional consequences of the BsmI, ApaI (both located in intron 8) and the TaqI (p.Ile352Ile) variants, which are known to be in strong linkage disequilibrium, are less clear, with conflicting reports of the effects of the most commonly found haplotypes on VDR expression, mRNA stability and protein activity published by a number of investigators (summarized in Uitterlinden *et al.*, 2004). However individually carriers of the homozygous wild type TaqI variant display lower levels of circulating active vitamin D (Hustmyer *et al.*, 1993; Ma *et al.*, 1998; Morrison *et al.*, 1994), and the BsmI variant may affect gene expression via regulation of mRNA stability (Jurutka *et al.*, 2001).

Association of these SNPs with melanoma risk has been investigated by a number of groups with somewhat variable results, as is the norm for relatively small candidate gene association studies. To date four meta-analyses of VDR polymorphisms in skin cancer have been reported (Gandini *et al.*, 2009b; Mocellin and Nitti, 2008; Orlow *et al.*, 2012; Randerson-Moor *et al.*, 2009) focusing primarily on limited subsets of the functional gene variants. In 2009 we reported a study of the six *VDR* SNPs detailed above (Randerson-Moor *et al.*, 2009) in which there was no significant evidence of an association between any of the SNPs and risk in 1028 population-ascertained cases and 402 controls from Leeds, UK. However in a second Leeds case-control study (299 cases and 560 controls) the FokI T allele was associated with increased melanoma risk (OR 1.42, 95% CI 1.06-1.91, P=0.02). In the accompanying meta-analysis performed in conjunction with published data from other smaller data sets (total 3769 cases and 3636 controls), the FokI T allele was associated with increased melanoma risk (OR 1.19, 95% CI 1.05-1.35), whilst the BsmI A allele was associated with a reduced risk (OR 0.81, 95% CI 0.72-0.92), in each instance under a parsimonious dominant model. A second meta-analysis of BsmI and FokI was reported concurrently in the same year (Gandini *et al.*, 2009b), which reported similar findings. Most recently a meta-analysis of GATA, BsmI and TaqI, which included data from an additional Polish case control series (Gapska *et al.*, 2009), showed a similar protective effect for BsmI to that observed in previous meta-analyses (Orlow *et al.*, 2012).

Genome wide association studies have identified a number of SNPs associated with serum levels of vitamin D located near genes involved in vitamin D synthesis, activation or transport; the strongest being an intronic SNP (rs2282679) in the gene coding for the vitamin D binding protein (Ahn *et al.*, 2010; Wang *et al.*, 2010) confirmed in replication cohorts. Large-scale studies of this SNP in a series of case-control studies are indicated to investigate whether this gene is likely to be implicated in melanoma risk.

These data would appear therefore to provide modest support for the hypothesis that vitamin D might be protective for melanoma if we accept the view that an association between genetic variation in the receptor to which it binds has a small (but potentially biologically significant) association with risk. The most recent data from the GEM study do however provide somewhat conflicting information, in that in this study in which individuals with a single melanoma primary were compared with those with multiple primaries, inheritance of the BsmI variant was associated with an increased risk of multiple primary tumours (Mandelcorn-Monson *et al.*, 2011). The comparison reported by the GEM group was however between single case and

multiple case individuals, so it is difficult to compare with the other comparisons of cases and controls. SNPs in genes in the vitamin D pathway have furthermore not so far been identified in genome wide association studies reported by a number of groups (Amos *et al.*, 2011; Barrett *et al.*, 2011; Bishop *et al.*, 2009; Gudbjartsson *et al.*, 2008; Macgregor *et al.*, 2011) as melanoma risk alleles, so although this does not exclude an association it suggests that any such association would be weak in magnitude.

22.6 Summary, vitamin D and melanoma risk

In summary then, there is no doubt that intermittent sun exposure such as that which pale-skinned people get on holiday is associated with melanoma risk, if that sun exposure causes sunburn. That outdoor workers in temperate climates are not at increased risk of melanoma and may even have a lower risk (Gandini *et al.*, 2005b), suggests a complex relationship between sun exposure and the aetiology of melanoma. Allied with recent findings of a protective effect of regular weekend sun exposure of 5 or so hours each day at the weekend in our case-control study in the UK, these observations suggest that regular moderate sun exposure might reduce risk and this could be related to higher levels of vitamin D (although photoadaption is another possible explanation). There are biological reasons to suppose that vitamin D production in the skin might control innate immunity and potentially mediate immunological responses to UV-induced damage and therefore that it might be protective for skin cancer. Furthermore vitamin D has demonstrable anti-proliferative effects on melanoma cells *in vitro* (Reichrath *et al.*, 2007b) so that vitamin D might be protective in terms of limiting growth of precursor lesions such as naevi. The immunological effects of vitamin D are however very complex and one could cite cogent arguments why its effects might actually have the reverse effect. The genetic association studies between polymorphisms in the gene coding for the vitamin D receptor and risk provide some support for a relationship but these data remain relatively weak currently.

22.7 Advice to the general public on prevention of melanoma and vitamin D

The data on vitamin D and risk of melanoma is interesting but immature and certainly too weak to support public health programmes suggesting that vitamin D supplementation should be considered to prevent melanoma.

The 'at risk' population for melanoma are however fair-skinned, and the majority easily burn in the sun. There is no doubt that sunburn causes melanoma, therefore in these people it is sensible to give the advice that they should limit their sun exposure to avoid burning. In the Leeds Melanoma Cohort we saw that those people (cases and controls) with sun-sensitive phenotypes actually spend less time in the sun than the non-sensitive (Newton-Bishop *et al.*, 2011) (presumably because of their tendency to burn), yet those phenotypes are associated with risk. Furthermore the vitamin D levels were lower in these people (Davies *et al.*, 2011) and this observation suggests that in practice it is difficult for the fair-skinned to avoid sunburn without becoming vitamin

D deficient. As low levels of vitamin D are associated with reduced bone health and there are some data suggestive of lower overall mortality with supplementation (Autier and Gandini, 2007) one can argue that the fair-skinned should avoid the sun and take supplementation to avoid sub-optimal vitamin D levels. The level of supplementation remains controversial and will be discussed below.

It will be important in public health campaigns to find a way of allowing individuals to understand the relative risk benefit for them of sun exposure related to their skin type; how their skin behaves in the sun. The balance of risk will also depend upon the intensity of sun exposure so that advice will depend upon latitude of residence. What is clear is that skin colour is the main determinant of that balance and we will have to learn how best to convey this effectively. Such issues have been explored in relation to New Zealand where the melanoma rates are the highest in the world and the population comprised of Maori and Pacific peoples, as well as those of European origin (Callister *et al.*, 2011).

22.8 Melanoma progression/prognosis and vitamin D

In 2005 Berwick *et al.* reported a better outcome for melanoma patients recruited to a case-control study in the US, in the presence of solar elastosis in the skin (Berwick *et al.*, 2005). Solar elastosis is histologically detected damage to the connective tissues in the dermis, which is seen in elderly skin on sun-exposed sites. The inference made was that sun exposure was associated with better survival in melanoma but that the observation might also be explained if biological differences between melanomas arising in chronically sun-exposed skin and other tumours might 'explain' different survival. This first finding however was of interest.

We subsequently reported data from the Leeds Melanoma Cohort which suggested that melanoma patients bled after diagnosis (we attempted to sample 3 to 6 months after diagnosis) who had higher levels of vitamin D had thinner tumours, and survival data corrected for thickness and other factors known to predict outcome, such as age and sex, showed an independent survival benefit for the higher vitamin D levels (Newton-Bishop *et al.*, 2009). There has been a small subsequent study which showed a protective effect in stage IV melanoma (Nurnberg *et al.*, 2009), but the lack of sufficiently large cohorts with stored serum has meant that there has, to date, been limited opportunity to look at this in other cohorts. The data must therefore be taken as un-validated. The literature furthermore contains several instances of failure of randomised clinical trials to validate strong observational evidence linking the use of vitamins to lower risk outcome in cancer. This was shown for vitamin E and beta-carotene and in meta-analysis there even appeared to be an increased mortality associated with supplementation (Fernandez *et al.*, 2012) thereby raising the possibility that vitamin levels might simply be markers of other determinants of risk, or even that they might have different effects in terms of primary prevention than secondary prevention. It does not seem inconceivable, for example, that antioxidants might be important in primary prevention but could impair survival for cancer patients if host defence mechanisms utilised oxidative stress.

Although there are *in vitro* data to suggest that vitamin D is antiproliferative for melanoma (Reichrath *et al.*, 2007a), and consequently there might be a valuable biological effect of vitamin D on melanoma, there are considerable difficulties in exploring this further. Vitamin D levels are known to be higher in people with lower body mass index (Brock *et al.*, 2010), and to be associated with more exercise and generally better health and therefore some have argued that the association between higher vitamin D levels and lower risk of many diseases might reflect a causal relationship, not between vitamin D and those diseases, but between other medical conditions associated with poor outcomes such as the metabolic syndrome. There seems to be a clear relationship between lower serum levels and increased mortality (discussed in Chapter 10 of this book) but the suggestion is that serum vitamin D levels could be associated here with outcome for melanoma just as markers of other causal aspects of health. The argument is rather circular, as others have reported evidence that low vitamin D levels might be a cause of the metabolic syndrome (Pacifico *et al.*, 2011), and a modest reversal of the metabolic syndrome phenotype has been demonstrated after supplementation (Al-Daghri *et al.*, 2011).

We have therefore explored Mendelian Randomization as a method of using measured variation in genes of known function (in this case the vitamin D binding protein) to examine the causal effect of a modifiable exposure, (that is serum vitamin D level). This was first described by Gray and Wheatley in 1991 as a method for obtaining unbiased estimates of the effects of a putative casual variable which they suggested might be achieved without conducting a traditional randomized trial (Gray and Wheatley, 1991). If the reported association between serum vitamin D levels and survival is actually a marker for some other more potent determinant, such as the metabolic syndrome, then the association between the genetic determinants of serum levels would show a different relationship to melanoma survival than do the serum levels. The genetic determinants of vitamin D level in the serum have been published from genome wide association studies, which showed the strongest effect for a SNP rs2282679 in the gene coding for the vitamin D binding protein (Ahn *et al.*, 2010; Wang *et al.*, 2010). We have subsequently reported a strong association between this SNP and lower levels of vitamin D (Davies *et al.*, 2011); dietary supplementation, holiday and regular weekend sun exposure, body mass index and skin type were also major determinants of serum levels (Davies *et al.*, 2011). We postulated that if inheritance of the SNP associated with lower levels of vitamin D was associated with poorer survival from melanoma then, using the principle of Mendelian Randomization, this would provide support for the hypothesis that vitamin D has a causal relationship with survival. These data are currently being generated.

There is an enormous literature on the association between serum vitamin D levels and cancer risk and an equally large literature of the biological effects of vitamin D on cancer cells. Certainly there are strong *in vitro* data to suggest that vitamin D is anti-proliferative for some but not all melanoma lines (Seifert *et al.*, 2004) which we have confirmed in our own laboratory using pharmacological doses of vitamin D. It is supposed that these anti-proliferative effects likely 'explain' the potential beneficial effects of vitamin D in melanoma, and that these effects would be mediated by the VDR. Expression of *VDR* mRNA corresponds to responsiveness to added vitamin D *in vitro* (Essa *et al.*, 2010) suggesting that the effects of vitamin D are mediated by

transcriptional changes in the cells. *In vivo* there is evidence that VDR expression diminishes with melanoma progression (Brozyna *et al.*, 2011) and that expression is particularly reduced in pigmented tumours. So that, in life at least, any putative protective effects of vitamin D in melanoma patients are likely to vary according to the genomic profile of the tumour.

There are further potential effects of vitamin D for cancer patients as the hormone has been reported to have anti-angiogenic effects (Iseki *et al.*, 1999; Shokravi *et al.*, 1995). Vitamin D is also thought to have a role in controlling excessive immune mediated inflammation, evidence coming from the susceptibility of VDR knockout mice to a type of inflammatory bowel disease (Soudja *et al.*, 2010) and evidence of inhibition of TH17 cells in a transgenic mouse, mediated by the VDR (Joshi *et al.*, 2011). Chronic inflammation is thought to be deleterious in melanomas, driven by cytokines produced by tumour cells and macrophages, and leading to angiogenesis and suppression of adaptive immunity, so that in an individual in which this was the dominant process one could hypothesise that increasing vitamin D levels might be beneficial. There are also data to suggest that vitamin D enhances natural killer cell killing of melanoma cells (Lee *et al.*, 2011) which might be beneficial too.

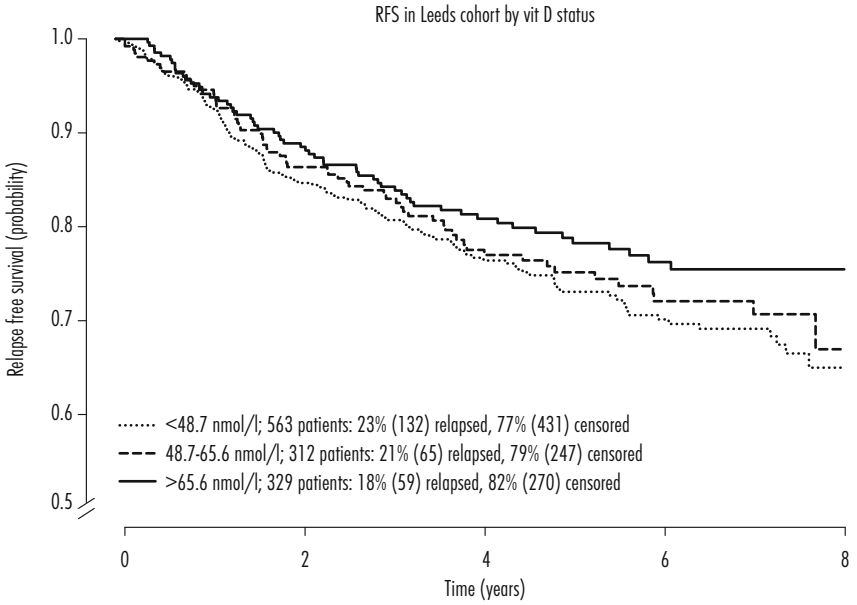

Figure 22.1. Kaplan Meier curve showing survival for 1,204 patients in the Leeds Melanoma Cohort by serum vitamin D level at recruitment to the study (levels were measured where possible 3 to 6 months after diagnosis). The levels are split into tertiles showing better survival for participants with serum vitamin D levels in excess of 65.6 nmol/l.

In the period since we reported the relationship between serum vitamin D levels and outcome in the Leeds Melanoma Cohort, the effects of vitamin D level around diagnosis on survival have persisted (Figure 22.1).

The role of vitamin D in survival for melanoma patients however remains unclear. There is clear evidence that the prevalence of suboptimal (even deficient) levels of serum vitamin D in the UK melanoma population is high at diagnosis. There is also evidence that sun avoidance is common after diagnosis (indeed dermatologists usually advise this) (Idorn *et al.*, 2011) so that the proportion of melanoma patients who are deficient is likely to increase after diagnosis.

We therefore took the view that it would be prudent to advise patients to be aware of this risk and to avoid further deficiency by compensatory ingestion of fortified foods or modest vitamin D supplements. However it is clear that there are potential ill effects for melanoma patients of higher vitamin D levels. There are data from breast cancer studies (Goodwin *et al.*, 2009) and cardiovascular disease (Wang *et al.*, 2008) of a U-shaped effect so that both low and high levels of vitamin D might be harmful. More recently too, data have been published in which CRP levels in a large sample set were used as a marker of a pro-inflammatory state and therefore increased risk of cardiovascular disease in otherwise healthy people, and was correlated with serum vitamin D levels (Amer and Qayyum, 2012). The lowest levels of CRP were actually associated with vitamin D levels around 52.5 nmol/l. There was a fall in CRP by a geometric mean of 0.285 mg/dl for every 25 nmol/l increase in serum vitamin D level up to 52.5 nmol/l but then a rise of 0.05 mg/dl for every 25 nmol/l of vitamin D above 52.2 nmol/l, suggesting again that optimal levels for some measures of health (other than for bone health) might be at the lower end of 'normal' levels previously reported. The observed CRP changes, used as a measure of chronic inflammation, suggest that there would be no benefit (and may even be a small adverse effect) of increasing serum levels of vitamin D above 52.5 nmol/l.

Of particular concern for melanoma patients is the complex relationship between vitamin D and immunity. Melanoma is an immunogenic tumour. Spontaneous regression of advanced disease has been described (albeit rarely). Tumour infiltrating lymphocytes are common in primary tumours and brisk infiltration carries a better prognosis (Elder and Murphy, 1991), the development of vitiligo (auto-immune mediated damage to melanocytes in the skin) during chemotherapy is a good prognostic sign. Most recently immunotherapy with antibodies to the immunosuppressive molecule CTLA4 have shown survival benefit from treated patients, even those patients with pre-treated stage IV melanoma (Robert *et al.*, 2011). It is clear that vitamin D has potent effects on the immune system but they are complex and there are concerns that for cancer patients some of those effects might be harmful if the net effect of increasing vitamin D levels is immunosuppression.

We could hypothesise that the effects of increasing vitamin D levels for melanoma patients would depend upon the relative activity of the innate versus adaptive immune systems. As discussed above the absence of the VDR in mice provides a very interesting model of inflammatory bowel disease (Froicu and Cantorna, 2007) in which the mice develop excessive uncontrolled

inflammation (mediated by innate immune systems) after minor bowel injury with high expression of inflammatory cytokines. Thus vitamin D may be a regulator of innate immunity.

Although the development of adaptive immunity appears to be beneficial for melanoma patients, in mice models tumour-initiated inflammation overrides the protective effects of adaptive immunity (Soudja *et al.*, 2010). Thus, whilst induction of innate immune-like responses using toll-like receptor agonists such as Aldara has been shown to be effective treatment of melanoma in the skin (Schon and Schon, 2007), protracted inflammation may be deleterious so that moderation of that chronic inflammation would likely be beneficial if indeed increasing vitamin D levels had such an effect.

Vitamin D however also appears to suppress adaptive immunity and it is thought that supplementation with vitamin D might be useful in autoimmunity by having such an effect (Zold *et al.*, 2008). Suppression of adaptive immunity in melanoma patients by whatever means would be inadvisable one would suppose, and this is the theoretical concern around supplementation in cancer patients.

The avoidance of 'high' serum levels of vitamin D would seem to be necessary. One of the fundamental difficulties however is that there is a lack of clarity as to what constitutes a 'high' level. Some argue that it's difficult to generate toxicity from vitamin D but hypercalcaemia may occur; indeed prevention trials have reported an increased risk of kidney stones in vitamin D treated patients, for example in the Women's Health Initiative Trial (Glendenning *et al.*, 2012). The usual lower end of the quoted 'optimal' blood range of serum vitamin D is 60 to 78 nmol/l summarised in a recent review (Field and Newton-Bishop, 2011). Toxicity is described as unlikely at under 325 nmol/l and although the upper range in many laboratories is around 125 nmol/l life-guards often have levels higher than this (Holick, 2004)

Parathyroid levels are high in the presence of low vitamin D levels but they plateau at serum levels of vitamin D of around 60 to 78 nmol/l although it is clear from a meta-analysis by Bjorkman that even this is complex (Bjorkman *et al.*, 2009). In our studies in which we reported the associations between serum levels, sun exposure, supplementation and inherited variation in genes reported to be associated with serum levels (reviewed by McGrath *et al.*, 2010), the median level of vitamin D also plateaued at 60 nmol/l (Davies *et al.*, 2011) with increased sun exposure (Figure 22.2). The observations made by Amer and Qayyum (2012), discussed above relating to CRP levels and serum vitamin D, and the suggestion of a U-shaped curve for protection from breast cancer deaths (Goodwin *et al.*, 2009) or cardiovascular disease (Wang *et al.*, 2008), to us at least suggest that for cancer and cardiovascular disease it may be that minimal serum levels of vitamin D of around 60 nmol/l might be preferable. It must be said that in our studies we have not seen any evience at all of a deleterious effect of higher vitamin D levels in the Leeds Melanoma Cohort but it is also true that levels above 100 nmol/l were rare. There is of course marked seasonal variation in levels and peak to trough seasonal variation has been reported to be around 25 nmol/l (Shoben *et al.*, 2011). We suggest therefore that we should consider aiming for levels in the range 60 to

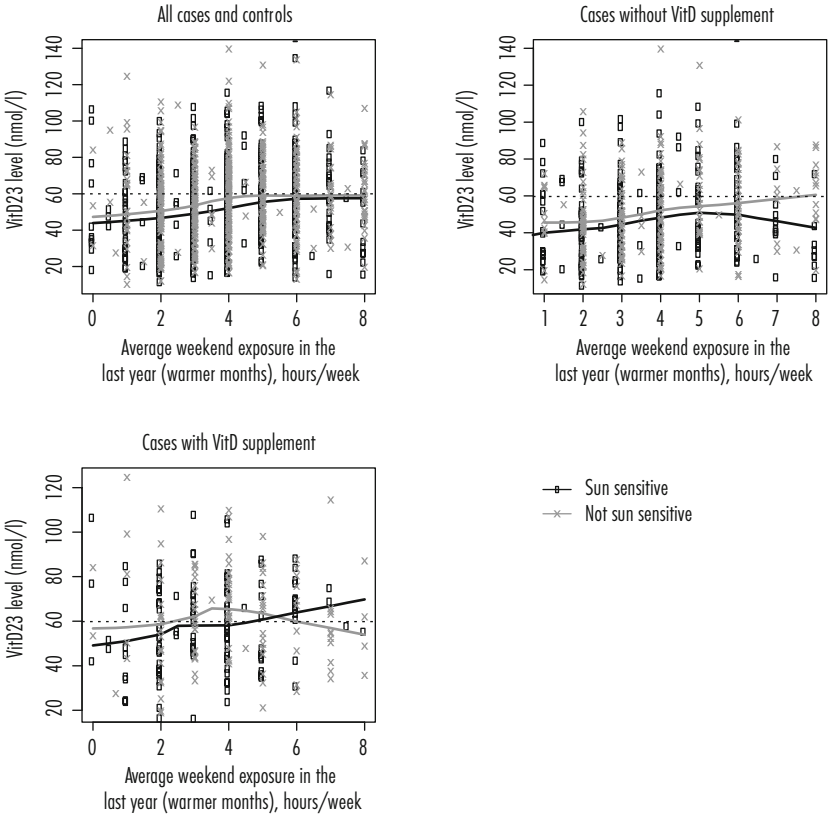

Figure 22.2. The median serum vitamin D levels achieved by participants in the Leeds Melanoma Cohort as a result of increasing numbers of hours spent outside at weekends in the warmer months (Davies *et al.*, 2011). The data are divided into those for individuals defined as sun sensitive and non-sun sensitive (on the basis of responses to questions about skin pigmentation and the effects of UV exposure). It can be seen that the sun-sensitive have median levels of vitamin D which are lower than those for the not sun-sensitive (hypothesised to be because the sun-sensitive find it difficult to stay outside in the sun for long enough without burning). It can also be seen that the median levels reach a plateau at around 60 nmol/l after 4 hours of daily weekend sun exposure for the not sun-sensitive and 6 hours for the sun-sensitive. Median levels of 60 nmol/l were reached in those taking supplements irrespective of sun exposure.

85 nmol/l throughout the year in cancer patients in the period before randomized clinical trials have been performed.

The literature suggested however that achieving this is likely to be difficult. Variation in sun exposure and behaviour in the sun will have a marked effect. There are also marked differences in how individuals respond to sunlight exposure and oral intake of vitamin D. Skin colour is a strong determinant. Melanoma patients tend to be white and the biggest differences are between black and white-skinned people but even in the white-skinned peoples there is variation. Three

studies have shown that the palest people have lower levels (Davies *et al.*, 2011; Glass *et al.*, 2009), presumably because in real life individuals who are sensitive to the sun find it difficult to be outside uncovered for long enough to produce sufficient vitamin D without burning (Figure 22.2). Levels tend to be lower in the obese and our own studies have suggested that inherited variation in the gene coding for the vitamin D binding protein determines serum levels of vitamin D achieved by increased sun exposure and supplementation (Figure 22.3). A review by Balk (2011) summarised the findings of studies designed to evaluate the supplementation required to maintain levels at 62.5 nmol/l in adults which ranged from 400 to around 1,500 IU per day.

We have measured vitamin D levels in melanoma patients in follow up in the UK and have experienced this variation. Some patients appear to increase their serum levels from less than 20 nmol/l to 70 nmol/l, for example, in 3 months with 1 cod liver oil capsule whereas others struggle to reach these levels using oral supplements in over 12 months. This has led many to give high loading doses to deficient patients and this might be preferable for cancer patients if we take the view, as some have done, that it makes sense to increase the levels as quickly as possible after cancer diagnoses. The published evidence of suppression of adaptive immunity by vitamin D however causes us concern about rapid changes of level.

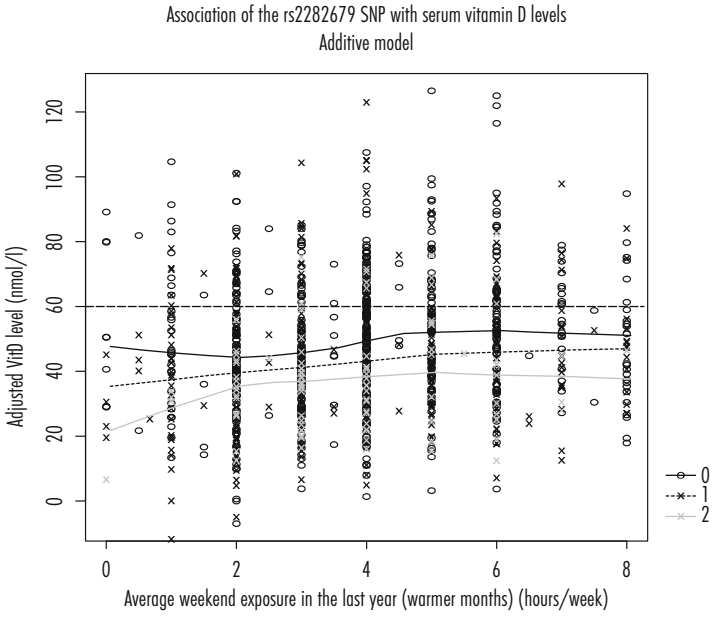

Figure 22.3. The median serum vitamin D levels achieved by participants in the Leeds Melanoma Cohort as a result of increasing numbers of hours spent outside at weekends in the warmer months (Davies *et al.*, 2011). The three curves indicate levels reached according to inheritance of the SNP rs2282699 in the gene coding for the vitamin D binding protein. Individuals homozygous for the SNP associated with lower levels of the protein run at significantly lower levels of vitamin D for similar periods of sun exposure.

Our personal view is that we should aim for levels of serum vitamin D in the range 60 to 85 nmol/l achieved by daily supplementation with vitamin D3 in the range 400 to 1000 IU per day depending on baseline levels of vitamin D at diagnosis. This advice is based upon a cautious view of the literature but fully cognisant that the necessity is to perform a randomised clinical trial.

The Italians have published a plan for such an adjuvant trial in stage 1 to 2 melanoma patients (Gandini *et al.*, 2009a) but we are not aware if it has started as yet. Whilst a randomized clinical trial is very desirable it would be a very difficult trial indeed to design. It is not clear what blood level we would aim for or how to achieve that level. It seems likely to us that responses to supplementation might depend furthermore on the status of host/tumour interaction at the time.

We have elected to continue to research the relationship between vitamin D and melanoma in order to inform the development of such a randomized trial, using genomics and by looking at serum samples in stored sample sets from clinical trials. In the meantime, in clinic we do not wish our melanoma patients to become even more vitamin D deficient after diagnosis than they were at presentation. We therefore gave general advice about avoiding supplementation but when we checked blood levels we found that the majority of our patients remained at low levels. We therefore now counsel:

- measurement of vitamin D levels in melanoma patients soon after diagnosis in the winter and summer;
- modest supplementation if levels are lower than 50 nmol/l depending on season;
- aiming for levels between 60 nmol/l and 85 nmol/l;
- that a patient who has had a poor prognosis tumour in the past but suffered no relapse has shown good evidence of effective host/tumour interaction and it might be prudent for them to avoid supplementation.

References

Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K.C., McCullough, M.L., Gallicchio, L., Jacobs, E.J., Ascherio, A., Helzlsouer, K., Jacobs, K.B., Li, Q., Weinstein, S.J., Purdue, M., Virtamo, J., Horst, R., Wheeler, W., Chanock, S., Hunter, D.J., Hayes, R.B., Kraft, P. and Albanes, D., 2010. Genome-wide association study of circulating vitamin D levels. Human Molecular Genetics 19, 2739-2745.

Al-Daghri, N.M., Alkharfy, K.M., Al-Saleh, Y., Al-Attas, O.S., Alokail, M.S., Al-Othman, A., Moharram, O., El-Kholie, E., Sabico, S., Kumar, S. and Chrousos, G.P., 2011. Modest reversal of metabolic syndrome manifestations with vitamin D status correction: a 12-month prospective study. Metabolism: Clinical and Experimental 61, 661-666.

Amer, M. and Qayyum, R., 2012. Relation between serum 25-hydroxyvitamin d and C-reactive protein in asymptomatic adults (from the continuous national health and nutrition examination survey 2001 to 2006). The American journal of cardiology 109, 226-230.

Amos, C.I., Wang, L.E., Lee, J.E., Gershenwald, J.E., Chen, W.V., Fang, S., Kosoy, R., Zhang, M., Qureshi, A.A., Vattathil, S., Schacherer, C.W., Gardner, J.M., Wang, Y., Tim Bishop, D., Barrett, J.H., Macgregor, S., Hayward, N.K., Martin, N.G., Duffy, D.L., Mann, G.J., Cust, A., Hopper, J., Brown, K.M., Grimm, E.A., Xu, Y., Han, Y., Jing, K., McHugh, C., Laurie, C.C., Doheny, K.F., Pugh, E.W., Seldin, M.F., Han, J. and Wei, Q., 2011. Genome-wide association study identifies novel loci predisposing to cutaneous melanoma. Human Molecular Genetics 20, 5012-5023.

Arai, H., Miyamoto, K.I., Yoshida, M., Yamamoto, H., Taketani, Y., Morita, K., Kubota, M., Yoshida, S., Ikeda, M., Watabe, F., Kanemasa, Y. and Takeda, E., 2001. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. Journal of Bone and Mineral Research 16, 1256-1264.

Autier, P. and Gandini, S., 2007. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Archives of Internal Medicine 167, 1730-1737.

Balk, S.J., 2011. Ultraviolet radiation: a hazard to children and adolescents. Pediatrics 127, e791-817.

Barrett, J.H., Iles, M.M., Harland, M., Taylor, J.C., Aitken, J.F., Andresen, P.A., Akslen, L.A., Armstrong, B.K., Avril, M.F., Azizi, E., Bakker, B., Bergman, W., Bianchi-Scarra, G., Bressac-de Paillerets, B., Calista, D., Cannon-Albright, L.A., Corda, E., Cust, A.E., Debniak, T., Duffy, D., Dunning, A.M., Easton, D.F., Friedman, E., Galan, P., Ghiorzo, P., Giles, G.G., Hansson, J., Hocevar, M., Hoiom, V., Hopper, J.L., Ingvar, C., Janssen, B., Jenkins, M.A., Jonsson, G., Kefford, R.F., Landi, G., Landi, M.T., Lang, J., Lubinski, J., Mackie, R., Malvehy, J., Martin, N.G., Molven, A., Montgomery, G.W., van Nieuwpoort, F.A., Novakovic, S., Olsson, H., Pastorino, L., Puig, S., Puig-Butille, J.A., Randerson-Moor, J., Snowden, H., Tuominen, R., Van Belle, P., van der Stoep, N., Whiteman, D.C., Zelenika, D., Han, J., Fang, S., Lee, J.E., Wei, Q., Lathrop, G.M., Gillanders, E.M., Brown, K.M., Goldstein, A.M., Kanetsky, P.A., Mann, G.J., Macgregor, S., Elder, D.E., Amos, C.I., Hayward, N.K., Gruis, N.A., Demenais, F., Bishop, J.A. and Bishop, D.T., 2011. Genome-wide association study identifies three new melanoma susceptibility loci. Nature Genetics 43, 1108-1113.

Berwick, M., Armstrong, B.K., Ben-Porat, L., Fine, J., Kricker, A., Eberle, C. and Barnhill, R., 2005. Sun exposure and mortality from melanoma. Journal of the National Cancer Institute 97, 195-199.

Bishop, D.T., Demenais, F., Iles, M.M., Harland, M., Taylor, J.C., Corda, E., Randerson-Moor, J., Aitken, J.F., Avril, M.F., Azizi, E., Bakker, B., Bianchi-Scarra, G., Bressac-de Paillerets, B., Calista, D., Cannon-Albright, L.A., Chin, A.W.T., Debniak, T., Galore-Haskel, G., Ghiorzo, P., Gut, I., Hansson, J., Hocevar, M., Hoiom, V., Hopper, J.L., Ingvar, C., Kanetsky, P.A., Kefford, R.F., Landi, M.T., Lang, J., Lubinski, J., Mackie, R., Malvehy, J., Mann, G.J., Martin, N.G., Montgomery, G.W., van Nieuwpoort, F.A., Novakovic, S., Olsson, H., Puig, S., Weiss, M., van Workum, W., Zelenika, D., Brown, K.M., Goldstein, A.M., Gillanders, E.M., Boland, A., Galan, P., Elder, D.E., Gruis, N.A., Hayward, N.K., Lathrop, G.M., Barrett, J.H. and Bishop, J.A., 2009. Genome-wide association study identifies three loci associated with melanoma risk. Nature Genetics 41, 920-925.

Bjorkman, M., Sorva, A. and Tilvis, R., 2009. Responses of parathyroid hormone to vitamin D supplementation: a systematic review of clinical trials. Archives of Gerontology and Geriatrics 48, 160-166.

Boonstra, A., Barrat, F.J., Crain, C., Heath, V.L., Savelkoul, H.F.J. and O'Garra, A., 2001. 1 alpha,25-dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. Journal of Immunology 167, 4974-4980.

Brock, K., Huang, W.Y., Fraser, D.R., Ke, L., Tseng, M., Stolzenberg-Solomon, R., Peters, U., Ahn, J., Purdue, M., Mason, R.S., McCarty, C., Ziegler, R.G. and Graubard, B., 2010. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. Journal of Steroid Biochemistry and Molecular Biology 121, 462-466.

Brozyna, A.A., Jozwicki, W., Janjetovic, Z. and Slominski, A.T., 2011. Expression of vitamin D receptor decreases during progression of pigmented skin lesions. Human Pathology 42, 618-631.

Callister, P., Galtry, J. and Didham, R., 2011. The risks and benefits of sun exposure: should skin colour or ethnicity be the main variable for communicating health promotion messages in New Zealand? Ethnicity & Health 16, 57-71.

Chang, Y.M., Barrett, J.H., Bishop, D.T., Armstrong, B.K., Bataille, V., Bergman, W., Berwick, M., Bracci, P.M., Elwood, J.M., Ernstoff, M.S., Gallagher, R.P., Green, A.C., Gruis, N.A., Holly, E.A., Ingvar, C., Kanetsky, P.A., Karagas, M.R., Lee, T.K., Le Marchand, L., Mackie, R.M., Olsson, H., Osterlind, A., Rebbeck, T.R., Sasieni, P., Siskind, V., Swerdlow, A.J., Titus-Ernstoff, L., Zens, M.S. and Newton-Bishop, J.A., 2009. Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. International Journal of Epidemiology 38, 814-830.

Colin, E.M., Weel, A.E.A.M., Uitterlinden, A.G., Buurman, C.J., Birkenhager, J.C., Pols, H.A.P., van Leeuwen, J.P.T.M. and van, L., 2000. Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D-3. Clinical Endocrinology 52, 211-216.

Davies, J.R., Chang, Y.M., Snowden, H., Chan, M., Leake, S., Karpavicius, B., Haynes, S., Kukalizch, K., Randerson-Moor, J., Elliott, F., Barth, J., Kanetsky, P.A., Harland, M., Bishop, D.T., Barrett, J.H. and Newton-Bishop, J.A., 2011. The determinants of serum vitamin D levels in participants in a melanoma case-control study living in a temperate climate. Cancer causes & control 22, 1471-1482.

Deeb, K.K., Trump, D.L. and Johnson, C.S., 2007. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nature Reviews: Cancer 7, 684-700.

Dixon, K.M., Sequeira, V.B., Camp, A.J. and Mason, R.S., 2010. Vitamin D-fence. Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology 9, 564-570.

Donawho, C.K. and Kripke, M.L., 1991. Evidence that the local effect of ultraviolet radiation on the growth of murine melanomas is immunologically mediated. Cancer Research 51, 4176-4181.

Downing, A., Newton-Bishop, J.A. and Forman, D., 2006. Recent trends in cutaneous malignant melanoma in the Yorkshire region of England; incidence, mortality and survival in relation to stage of disease, 1993-2003. British Journal of Cancer 95, 91-95.

Duffy, D.L., Iles, M.M., Glass, D., Zhu, G., Barrett, J.H., Hoiom, V., Zhao, Z.Z., Sturm, R.A., Soranzo, N., Hammond, C., Kvaskoff, M., Whiteman, D.C., Mangino, M., Hansson, J., Newton-Bishop, J.A., Bataille, V., Hayward, N.K., Martin, N.G., Bishop, D.T., Spector, T.D. and Montgomery, G.W., 2010. IRF4 variants have age-specific effects on nevus count and predispose to melanoma. American Journal of Human Genetics 87, 6-16.

Easton, D., Cox, G., Macdonald, A. and Ponder, B., 1991. Genetic susceptibility to naevi- a twin study. British Journal of Cancer 64, 1164-1167.

Elder, D. and Murphy, G., 1991. Malignant tumors (melanomas and related lesions). Atlas of Tumor Pathology: Melanocytic Tumors of the Skin 2 (third series), 103-205.

Essa, S., Denzer, N., Mahlknecht, U., Klein, R., Collnot, E.M., Tilgen, W. and Reichrath, J., 2010. VDR microRNA expression and epigenetic silencing of vitamin D signaling in melanoma cells. Journal of Steroid Biochemistry and Molecular Biology 121, 110-113.

Falchi, M., Bataille, V., Hayward, N.K., Duffy, D.L., Bishop, J.A., Pastinen, T., Cervino, A., Zhao, Z.Z., Deloukas, P., Soranzo, N., Elder, D.E., Barrett, J.H., Martin, N.G., Bishop, D.T., Montgomery, G.W. and Spector, T.D., 2009. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. Nature Genetics 41, 915-919.

Fernandez, A.A., Paniker, L., Garcia, R. and Mitchell, D.L., 2012. Recent advances in sunlight-induced carcinogenesis using the Xiphophorus melanoma model. Comparative biochemistry and physiology. Toxicology & pharmacology : CBP 155, 64-70.

Field, S. and Newton-Bishop, J.A., 2011. Melanoma and vitamin D. Molecular Oncology 5, 197-214.

Fisher, M.S. and Kripke, M.L., 1977. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proceedings of the National Academy of Sciences of the USA 74, 1688-1692.

Fisher, M.S. and Kripke, M.L., 2002. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. 1977. Bulletin of the World Health Organization 80, 908-912.

Froicu, M. and Cantorna, M.T., 2007. Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. BMC Immunology 8, 5.

Gandini, S., Francesco, F., Johanson, H., Bonanni, B. and Testori, A., 2009a. Why vitamin D for cancer patients? Ecancermedicalscience 3, 160.

Gandini, S., Raimondi, S., Gnagnarella, P., Dore, J.F., Maisonneuve, P. and Testori, A., 2009b. Vitamin D and skin cancer: a meta-analysis. European Journal of Cancer 45, 634-641.

Gandini, S., Sera, F., Cattaruzza, M.S., Pasquini, P., Abeni, D., Boyle, P. and Melchi, C.F., 2005a. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. European Journal of Cancer 41, 28-44.

Gandini, S., Sera, F., Cattaruzza, M.S., Pasquini, P., Picconi, O., Boyle, P. and Melchi, C.F., 2005b. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. European Journal of Cancer 41, 45-60.

Gandini, S., Sera, F., Cattaruzza, M.S., Pasquini, P., Zanetti, R., Masini, C., Boyle, P. and Melchi, C.F., 2005c. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. European Journal of Cancer 41, 2040-2059.

Gapska, P., Scott, R.J., Serrano-Fernandez, P., Mirecka, A., Rassoud, I., Gorski, B., Cybulski, C., Huzarski, T., Byrski, T., Nagay, L., Maleszka, R., Sulikowski, M., Lubinski, J. and Debniak, T., 2009. Vitamin D receptor variants and the malignant melanoma risk: a population-based study. Cancer Epidemiology 33, 103-107.

Glass, D., Lens, M., Swaminathan, R., Spector, T.D. and Bataille, V., 2009. Pigmentation and vitamin D metabolism in Caucasians: low vitamin D serum levels in fair skin types in the UK. PloS One 4, e6477.

Glendenning, P., Zhu, K., Inderjeeth, C., Howat, P., Lewis, J.R. and Prince, R.L., 2012. Effects of three monthly oral 150,000 IU cholecalciferol supplementation on falls, mobility and muscle strength in older postmenopausal women: a randomised controlled trial. Journal of Bone and Mineral Research 27(1), 170-176.

Goodwin, P.J., Ennis, M., Pritchard, K.I., Koo, J. and Hood, N., 2009. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. Journal of Clinical Oncology 27, 3757-3763.

Gray, R. and Wheatley, K., 1991. How to avoid bias when comparing bone marrow transplantation with chemotherapy. Bone Marrow Transplantation 7 Suppl 3, 9-12.

Gudbjartsson, D.F., Sulem, P., Stacey, S.N., Goldstein, A.M., Rafnar, T., Sigurgeirsson, B., Benediktsdottir, K.R., Thorisdottir, K., Ragnarsson, R., Sveinsdottir, S.G., Magnusson, V., Lindblom, A., Kostulas, K., Botella-Estrada, R., Soriano, V., Juberias, P., Grasa, M., Saez, B., Andres, R., Scherer, D., Rudnai, P., Gurzau, E., Koppova, K., Kiemeney, L.A., Jakobsdottir, M., Steinberg, S., Helgason, A., Gretarsdottir, S., Tucker, M.A., Mayordomo, J.I., Nagore, E., Kumar, R., Hansson, J., Olafsson, J.H., Gulcher, J., Kong, A., Thorsteinsdottir, U. and Stefansson, K., 2008. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. Nature Genetics 40, 886-891.

Halsall, J.A., Osborne, J.E., Potter, L., Pringle, J.H. and Hutchinson, P.E., 2004. A novel polymorphism in the 1A promoter region of the vitamin D receptor is associated with altered susceptibilty and prognosis in malignant melanoma. British Journal of Cancer 91, 765-770.

Holick, M.F., 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. American Journal of Clinical Nutrition 80, 1678S-1688S.

Hustmyer, F.G., DeLuca, H.F. and Peacock, M., 1993. ApaI, BsmI, EcoRV and TaqI polymorphisms at the human vitamin D receptor gene locus in Caucasians, blacks and Asians. Human Molecular Genetics 2, 487.

Idorn, L.W., Philipsen, P.A. and Wulf, H.C., 2011. Sun exposure before and after a diagnosis of cutaneous malignant melanoma: estimated by developments in serum vitamin D, skin pigmentation and interviews. The British Journal of Dermatology 165, 164-170.

Iseki, K., Tatsuta, M., Uehara, H., Iishi, H., Yano, H., Sakai, N. and Ishiguro, S., 1999. Inhibition of angiogenesis as a mechanism for inhibition by 1alpha-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 of colon carcinogenesis induced by azoxymethane in Wistar rats. International journal of cancer. Journal International du Cancer 81, 730-733.

Joshi, S., Pantalena, L.C., Liu, X.K., Gaffen, S.L., Liu, H., Rohowsky-Kochan, C., Ichiyama, K., Yoshimura, A., Steinman, L., Christakos, S. and Youssef, S., 2011. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. Molecular and Cellular Biology 31, 3653-3669.

Jurutka, P.W., Remus, L.S., Whitfield, G.K., Thompson, P.D., Hsieh, J.C., Zitzer, H., Tavakkoli, P., Galligan, M.A., Dang, H.T.L., Haussler, C.A. and Haussler, M.R., 2000. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Molecular Endocrinology 14, 401-420.

Jurutka, P.W., Whitfield, G.K., Hsieh, J.C., Thompson, P.D., Haussler, C.A. and Haussler, M.R., 2001. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. Reviews in Endocrine & Metabolic Disorders 2, 203-216.

Lee, J.H., Park, S., Cheon, S., Kim, S., Hur, D.Y., Kim, T.S., Yoon, S.R., Yang, Y., Bang, S.I., Park, H., Lee, H.T. and Cho, D., 2011. 1,25-Dihydroxyvitamin D enhances NK susceptibility of human melanoma cells via Hsp60-mediated FAS expression. European Journal of Immunology 41, 2937-2946.

Ma, J., Stampfer, M.J., Gann, P.H., Hough, H.L., Giovannucci, E., Kelsey, K.T., Hennekens, C.H. and Hunter, D.J., 1998. Vitamin D receptor polymorphisms, circulating vitamin D metabolites, and risk of prostate cancer in United States physicians. Cancer Epidemiology, Biomarkers & Prevention 7, 385-390.

Macgregor, S., Montgomery, G.W., Liu, J.Z., Zhao, Z.Z., Henders, A.K., Stark, M., Schmid, H., Holland, E.A., Duffy, D.L., Zhang, M., Painter, J.N., Nyholt, D.R., Maskiell, J.A., Jetann, J., Ferguson, M., Cust, A.E., Jenkins, M.A., Whiteman, D.C., Olsson, H., Puig, S., Bianchi-Scarra, G., Hansson, J., Demenais, F., Landi, M.T., Debniak, T., Mackie, R., Azizi, E., Bressac-de Paillerets, B., Goldstein, A.M., Kanetsky, P.A., Gruis, N.A., Elder, D.E.,

Newton-Bishop, J.A., Bishop, D.T., Iles, M.M., Helsing, P., Amos, C.I., Wei, Q., Wang, L.E., Lee, J.E., Qureshi, A.A., Kefford, R.F., Giles, G.G., Armstrong, B.K., Aitken, J.F., Han, J., Hopper, J.L., Trent, J.M., Brown, K.M., Martin, N.G., Mann, G.J. and Hayward, N.K., 2011. Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3. Nature Genetics 43(11), 1114-1118.

Mandelcorn-Monson, R., Marrett, L., Kricker, A., Armstrong, B.K., Orlow, I., Goumas, C., Paine, S., Rosso, S., Thomas, N., Millikan, R.C., Pole, J.D., Cotignola, J., Rosen, C., Kanetsky, P.A., Lee-Taylor, J., Begg, C.B. and Berwick, M., 2011. Sun exposure, vitamin D receptor polymorphisms FokI and BsmI and risk of multiple primary melanoma. Cancer Epidemiology 35(6), e105-e110.

McGill, G.G., Horstmann, M., Widlund, H.R., Du, J., Motyckova, G., Nishimura, E.K., Lin, Y.L., Ramaswamy, S., Avery, W., Ding, H.F., Jordan, S.A., Jackson, I.J., Korsmeyer, S.J., Golub, T.R. and Fisher, D.E., 2002. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell 109, 707-718.

McGrath, J.J., Saha, S., Burne, T.H. and Eyles, D.W., 2010. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. The Journal of Steroid Biochemistry and Molecular Biology 121, 471-477.

Miller, J. and Gallo, R.L., 2010. Vitamin D and innate immunity. Dermatologic Therapy 23, 13-22.

Mocellin, S. and Nitti, D., 2008. Vitamin D receptor polymorphisms and the risk of cutaneous melanoma: a systematic review and meta-analysis. Cancer 113, 2398-2407.

Morrison, N.A., Qi, J.C., Tokita, A., Kelly, P.J., Crofts, L., Nguyen, T.V., Sambrook, P.N. and Eisman, J.A., 1994. Prediction of bone-density from vitamin-D receptor alleles. Nature 367, 284-287.

Newton-Bishop, J.A., Beswick, S., Randerson-Moor, J., Chang, Y.M., Affleck, P., Elliott, F., Chan, M., Leake, S., Karpavicius, B., Haynes, S., Kukalizch, K., Whitaker, L., Jackson, S., Gerry, E., Nolan, C., Bertram, C., Marsden, J., Elder, D.E., Barrett, J.H. and Bishop, D.T., 2009. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. Journal of Clinical Oncology 27, 5439-5444.

Newton-Bishop, J.A., Chang, Y.M., Elliott, F., Chan, M., Leake, S., Karpavicius, B., Haynes, S., Fitzgibbon, E., Kukalizch, K., Randerson-Moor, J., Elder, D.E., Bishop, D.T. and Barrett, J.H., 2011. Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. European Journal of Cancer 47, 732-741.

Newton-Bishop, J.A., Chang, Y.M., Iles, M.M., Taylor, J.C., Bakker, B., Chan, M., Leake, S., Karpavicius, B., Haynes, S., Fitzgibbon, E., Elliott, F., Kanetsky, P.A., Harland, M., Barrett, J.H. and Bishop, D.T., 2010. Melanocytic nevi, nevus genes, and melanoma risk in a large case-control study in the United Kingdom. Cancer Epidemiology, Biomarkers & Prevention 19, 2043-2054.

Noonan, F.P., Dudek, J., Merlino, G. and De Fabo, E.C., 2003. Animal models of melanoma: an HGF/SF transgenic mouse model may facilitate experimental access to UV initiating events. Pigment Cell Research 16, 16-25.

Norval, M., 2011. The challenges of UV-induced immunomodulation for children's health. Progress in Biophysics and Molecular Biology 107, 323-332.

Norval, M. and Wulf, H.C., 2009. Does chronic sunscreen use reduce vitamin D production to insufficient levels? British Journal of Dermatology 161, 732-736.

Nurnberg, B., Graber, S., Gartner, B., Geisel, J., Pfohler, C., Schadendorf, D., Tilgen, W. and Reichrath, J., 2009. Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients. Anticancer Research 29, 3669-3674.

Orlow, I., Roy, P., Reiner, A.S., Yoo, S., Patel, H., Paine, S., Armstrong, B.K., Kricker, A., Marrett, L.D., Millikan, R.C., Thomas, N.E., Gruber, S.B., Anton-Culver, H., Rosso, S., Gallagher, R.P., Dwyer, T., Kanetsky, P.A., Busam, K., From, L., Begg, C.B. and Berwick, M., 2012. Vitamin D receptor polymorphisms in patients with cutaneous melanoma. Journal International du Cancer 130, 405-418.

Pacifico, L., Anania, C., Osborn, J.F., Ferraro, F., Bonci, E., Olivero, E. and Chiesa, C., 2011. Low 25(OH)D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. European Journal of Endocrinology 165, 603-611.

Ramagopalan, S.V., Heger, A., Berlanga, A.J., Maugeri, N.J., Lincoln, M.R., Burrell, A., Handunnetthi, L., Handel, A.E., Disanto, G., Orton, S.M., Watson, C.T., Morahan, J.M., Giovannoni, G., Ponting, C.P., Ebers, G.C. and Knight, J.C., 2010. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Research 20, 1352-1360.

Randerson-Moor, J.A., Taylor, J.C., Elliott, F., Chang, Y.M., Beswick, S., Kukalizch, K., Affleck, P., Leake, S., Haynes, S., Karpavicius, B., Marsden, J., Gerry, E., Bale, L., Bertram, C., Field, H., Barth, J.H., Silva Idos, S., Swerdlow, A., Kanetsky, P.A., Barrett, J.H., Bishop, D.T. and Bishop, J.A., 2009. Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. European Journal of Cancer 45, 3271-3281.

Reichrath, J., Rech, M., Moeini, M., Meese, E., Tilgen, W. and Seifert, M., 2007. *In vitro* comparison of the vitamin D endocrine system in 1,25(OH)2D3-responsive and -resistant melanoma cells. Cancer Biology & Therapy 6, 48-55.

Rengarajan, J., Szabo, S.J. and Glimcher, L.H., 2000. Transcriptional regulation of Th1/Th2 polarization. Immunology Today 21, 479-483.

Robert, C., Thomas, L., Bondarenko, I., O'Day, S., M, D.J., Garbe, C., Lebbe, C., Baurain, J.F., Testori, A., Grob, J.J., Davidson, N., Richards, J., Maio, M., Hauschild, A., Miller Jr., W.H., Gascon, P., Lotem, M., Harmankaya, K., Ibrahim, R., Francis, S., Chen, T.T., Humphrey, R., Hoos, A. and Wolchok, J.D., 2011. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. The New England Journal of Medicine 364, 2517-2526.

Schon, M.P. and Schon, M., 2007. Imiquimod: mode of action. The British Journal of Dermatology 157 Suppl 2, 8-13.

Seifert, M., Rech, M., Meineke, V., Tilgen, W. and Reichrath, J., 2004. Differential biological effects of 1,25-dihydroxyVitamin D3 on melanoma cell lines *in vitro*. Journal of Steroid Biochemistry and Molecular Biology 89-90, 375-379.

Shoben, A.B., Kestenbaum, B., Levin, G., Hoofnagle, A.N., Psaty, B.M., Siscovick, D.S. and de Boer, I.H., 2011. Seasonal variation in 25-hydroxyvitamin D concentrations in the cardiovascular health study. American Journal of Epidemiology 174, 1363-1372.

Shokravi, M.T., Marcus, D.M., Alroy, J., Egan, K., Saornil, M.A. and Albert, D.M., 1995. Vitamin D inhibits angiogenesis in transgenic murine retinoblastoma. Investigative Ophthalmology & Visual Science 36, 83-87.

Soudja, S.M., Wehbe, M., Mas, A., Chasson, L., de Tenbossche, C.P., Huijbers, I., Van den Eynde, B. and Schmitt-Verhulst, A.M., 2010. Tumor-initiated inflammation overrides protective adaptive immunity in an induced melanoma model in mice. Cancer Research 70, 3515-3525.

Tang, J.Y., Fu, T., Leblanc, E., Manson, J.E., Feldman, D., Linos, E., Vitolins, M.Z., Zeitouni, N.C., Larson, J. and Stefanick, M.L., 2011. Calcium plus vitamin D supplementation and the risk of nonmelanoma and melanoma skin cancer: post hoc analyses of the women's health initiative randomized controlled trial. Journal of Clinical Oncology 29, 3078-3084.

Uitterlinden, A.G., Fang, Y., van Meurs, J.B.J., Pols, H.A.P. and van Leeuwen, J.P.T.M., 2004. Genetics and biology of vitamin D receptor polymorphisms. Gene 338, 143-156.

Valverde, P., Healy, E., Sikkink, S., Haldane, F., Thody, A.J., Carothers, A., Jackson, I.J. and Rees, J.L., 1996. The Asp84Glu variant of the melanocortin 1 receptor (MC1R) is associated with melanoma. Human Molecular Genetics 5, 1663-1666.

Vinceti, M., Malagoli, C., Fiorentini, C., Longo, C., Crespi, C.M., Albertini, G., Ricci, C., Lanzoni, A., Reggiani, M., Virgili, A., Osti, F., Lombardi, M., Santini, M., Fanti, P.A., Dika, E., Sieri, S., Krogh, V., Seidenari, S. and Pellacani, G., 2011. Inverse association between dietary vitamin D and risk of cutaneous melanoma in a northern Italy population. Nutrition and Cancer 63, 506-513.

Wachsmuth, R.C., Gaut, R.M., Barrett, J.H., Saunders, C.L., Randerson-Moor, J.A., Eldridge, A., Martin, N.G., Bishop, T.D. and Newton Bishop, J.A., 2001. Heritability and gene-environment interactions for melanocytic nevus density examined in a U.K. adolescent twin study. Journal of Investigative Dermatology 117, 348-352.

Wachsmuth, R.C., Turner, F., Barrett, J.H., Gaut, R., Randerson-Moor, J.A., Bishop, D.T. and Bishop, J.A., 2005. The effect of sun exposure in determining nevus density in UK adolescent twins. Journal of Investigative Dermatology 124, 56-62.

Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M. and Vasan, R.S., 2008. Vitamin D deficiency and risk of cardiovascular disease. Circulation 117, 503-511.

Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., van Meurs, J.B., Berry, D., Kiel, D.P., Streeten, E.A., Ohlsson, C., Koller, D.L., Peltonen, L., Cooper, J.D., O'Reilly, P.F., Houston, D.K., Glazer, N.L., Vandenput, L., Peacock, M., Shi, J., Rivadeneira, F., McCarthy, M.I., Anneli, P., de Boer, I.H., Mangino, M., Kato, B., Smyth, D.J., Booth, S.L., Jacques, P.F., Burke, G.L., Goodarzi, M., Cheung, C.L., Wolf, M., Rice, K., Goltzman, D., Hidiroglou, N., Ladouceur, M., Wareham, N.J., Hocking, L.J., Hart, D., Arden, N.K., Cooper, C., Malik, S., Fraser, W.D., Hartikainen, A.L., Zhai, G., Macdonald, H.M., Forouhi, N.G., Loos, R.J., Reid, D.M., Hakim, A., Dennison, E., Liu, Y., Power, C., Stevens, H.E., Jaana, L., Vasan, R.S., Soranzo, N., Bojunga, J., Psaty, B.M., Lorentzon, M., Foroud, T., Harris, T.B., Hofman, A., Jansson, J.O., Cauley, J.A., Uitterlinden, A.G., Gibson, Q., Jarvelin, M.R., Karasik, D., Siscovick, D.S., Econs, M.J., Kritchevsky, S.B., Florez, J.C., Todd, J.A., Dupuis, J., Hypponen, E. and Spector, T.D., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180-188.

Whiteman, D.C., Watt, P., Purdie, D.M., Hughes, M.C., Hayward, N.K. and Green, A.C., 2003. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. Journal of the National Cancer Institute 95, 806-812.

Whitfield, G.K., Remus, L.S., Jurutka, P.W., Zitzer, H., Oza, A.K., Dang, H.T.L., Haussler, C.A., Galligan, M.A., Thatcher, M.L., Dominguez, C.E. and Haussler, M.R., 2001. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Molecular and Cellular Endocrinology 177, 145-159.

Zaidi, M.R., Davis, S., Noonan, F.P., Graff-Cherry, C., Hawley, T.S., Walker, R.L., Feigenbaum, L., Fuchs, E., Lyakh, L., Young, H.A., Hornyak, T.J., Arnheiter, H., Trinchieri, G., Meltzer, P.S., De Fabo, E.C. and Merlino, G., 2011. Interferon-gamma links ultraviolet radiation to melanomagenesis in mice. Nature 469, 548-553.

Zold, E., Szodoray, P., Gaal, J., Kappelmayer, J., Csathy, L., Gyimesi, E., Zeher, M., Szegedi, G. and Bodolay, E., 2008. Vitamin D deficiency in undifferentiated connective tissue disease. Arthritis Research & Therapy 10, R123.

Key facts

- Prostate cancer is the most diagnosed cancer in men.
- Low sunlight exposure is a risk for prostate cancer.
- Vitamin D has functions other than maintaining good bones.
- Prostate has the ability to produce its own active form of vitamin D.
- Inhibitors of the enzyme which destroys the active form of vitamin D have potential to treat prostate cancer.

Summary points

- There is biochemical, ecological and epidemiological evidence for the connection between vitamin D and prostate cancer.
- Six cytochrome P450 enzymes have been implicated in the hydroxylation of vitamin D to 25(OH)D: CYP2C11, CYP27A1, CYP3A4, CYP2J3, CYP2D25 and CYP2R1. The prostate expresses CYP2R1, CYP27B1 and CYP24A1.
- CYP2R1 is expressed more in the prostate than in the liver, and can be down-regulated by 1a,25$(OH)_2$D3.
- The epidermal growth factor may play an important role in the development of prostate cancer and CYP27B1 is likely a tumor suppressor in the prostate.
- CYP24A1 is responsible for the hydroxylation at C-24 of 25(OH)D and 1a,25$(OH)_2$D, serving as the initial step to inactivate 1a,25$(OH)_2$D.
- MART-10 has shown to be more active in inhibiting prostate cell proliferation and is less susceptible to CYP24A1 degradation.
- Inhibitors of CYP24A1, such as VIMI and CPA1, decrease the degradation of the active hormone. CPA1 shows an 80-1 selective inhibition of CYP24A1 over CYP27B1.

23. Vitamin D autocrine system and prostate cancer prevention and treatment

T.C. Chen
Boston University School of Medicine, 715 Albany Street, Boston MA, 02118 USA;
taichen@bu.edu

Abstract

Cytochrome P450 (CYP) enzymes play crucial roles in vitamin D metabolism and actions, including hepatic vitamin D-25-hydroxylase and renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) for the synthesis of the active form 1α,25-dihydroxyvitamin D3 (1α,25$(OH)_2$D3), and 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) also in the kidneys to inactive 1α,25$(OH)_2$D3. The active hormone has high affinity for and interacts with vitamin D receptor (VDR), a transcription factor, to induce anti-proliferative, anti-invasive, anti-angiogenic, anti-inflammatory, pro-apoptotic and pro-differentiation actions in prostate cancer cells. However, it is now recognized that CYP27B1 and CYP24A1 are also expressed in many tissues and cells, including the prostate. Although six CYP enzymes have been identified with 25-hydroxylase activity, the two major ones are CYP27A1 and CYP2R1, and both are expressed in the prostate with CYP2R1 as the main 25-hydroxylase. The finding demonstrates that prostate tissue has the ability to activate and inactivate vitamin D in an autocrine/paracrine fashion. Recently 25-hydroxyvitamin D3 (25(OH)D3) and its analogs, without converting to their 1α-hydroxylated counter parts, have been shown to bind to VDR as agonists to modulate various gene expressions that ultimately lead to cell growth arrest and other anti-tumor activities. The finding suggests that the circulating levels of 25(OH)D, and the autocrine synthesis of 25(OH)D3 and 1α,25$(OH)_2$D3 may play an important role in regulating the growth of prostate cancer. Thus, in addition to 1α,25$(OH)_2$D3 analogs, the presence of CYP2R1, CYP27B1 and CYP24A1 in the prostate suggests that the analogs of vitamin D3 and 25(OH)D3, especially those that are resistant to CYP24A1 degradation, can be developed and used for the prevention and treatment of prostate cancer.

Keywords: vitamin D analog, proliferation, CYP enzymes, prostate cancer

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_23, © Wageningen Academic Publishers 2013

Abbreviation

1α,25(OH)$_2$D	1α,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
25-OHase	Vitamin D-25-hydroxylase
CYP450	Cytochrome P450
EGF	Epidermal growth factor
MAPK	Mitogen activated protein kinase
MAPKK	Mitogen activated protein kinase kinase
MART-10	19-nor-2α-(3-hydroxypropyl)-1α,25(OH)$_2$D3
PTH	Parathyroid hormone
UVB	Ultraviolet B
VDR	Vitamin D receptor
VDRE	Vitamin D response element

23.1 Introduction

There are six forms of naturally occurring vitamin D, namely, vitamin D2, vitamin D3, vitamin D4, vitamin D5, vitamin D6, and vitamin D7, with the same characteristic seco-steroid ring structure. Among them, vitamin D2 and vitamin D3 are the most important. Vitamin D2 or ergocalciferol is derived from fungi and plants, whereas vitamin D3 or cholecalciferol is produced in animal skin, after UVB (290-315 nm) irradiation (Holick *et al.*, 1980). In this chapter, vitamin D is used as a general term for both vitamin D2 and vitamin D3. The modern vitamin D research began in the mid 1960's, when vitamin D was found not biologically active (Norman *et al.*, 1964) requiring two successive hydroxylations, first at C-25 by 25-hydroxylases in the liver to produce 25(OH)D, and then at the C-1 position by 1α-hydroxylase in the kidneys to form 1α,25(OH)$_2$D, the active form of vitamin D (DeLuca, 1976). There is another important enzyme in the kidneys which can hydroxylate 1α,25(OH)$_2$D to 1α,24,25(OH)$_3$D or 25(OH)D to 24,25(OH)$_2$D as the first step of catabolic pathway to degrade 1α,25(OH)$_2$D or 25(OH)D (DeLuca, 1976; Schuster, 2011). 1α,25(OH)$_2$D is now believed to regulate more than 200 genes (Ramagopalan *et al.*, 2010) through its binding to VDR, including those involved in calcium and phosphorus metabolism, immune regulation, cell growth, cell differentiation, angiogenesis, inflammation and apoptosis. VDR has been identified in numerous cell types including prostate cells.

Prostate cancer is the most diagnosed and the second most fatal cancer among men in the US and Northern Europe. In the US, an estimate of 242,740 new cases of prostate cancer will be diagnosed and 28,170 will die from this disease in 2012 (Siegel *et al.*, 2012). Although the etiology of prostate cancer is not completely understood, ecological studies have repeatedly demonstrated the inversed relationship between sunlight (UVB) exposure and the incidence of prostate cancer (Grant, 2012; Rhee *et al.*, 2009), suggesting one cause of prostate cancer might be vitamin D insufficiency (Schwartz and Hulka, 1990). However, the direct epidemiological evidence to demonstrate the association of low serum 25-hydroxyvitamin D (25(OH)D) levels

with prostate cancer has been less consistent. The discrepancy among different reports may implicate the importance of the intraprostate concentrations of 25(OH)D and its ability to be activated rather than the serum levels of 25(OH)D as the risk factor for prostate cancer. In addition, the luck of association may result from different inclusive and exclusive criteria for the study subject selection and the time serum samples were obtained and how samples were stored and analyzed. Unlike the epidemiological studies, the biochemical evidence to support a role for vitamin D in prostate cancer is overwhelming (Chen and Holick, 2003; Miller, 1998; Swami *et al.*, 2011). VDR has been demonstrated in cultured prostate cells derived from non-cancerous and cancerous prostate tissues. 1α,25$(OH)_2$D and its analogs have been shown to inhibit prostate cell proliferation and invasion, and to stimulate prostate cell differentiation and apoptosis *in vitro* and tumor progression in animal models (Chen and Holick, 2003; Miller, 1998; Swami *et al.*, 2011). In this chapter, I will summarize the recent advances in the three enzymes involved in the activation and de-activation of vitamin D within the prostate cells and their implications in the prevention and treatment of prostate cancer.

23.2 Overview of human cytochrome P450 system

Cytochrome P450 enzymes belong to a family of isoenzymes which represents one of the largest and oldest gene superfamilies. This family of enzymes calalyzes the metabolism of a large number of compounds of both exogenous and endogenous origins, including drug detoxification and other xenobiotic metabolism, the metabolism of eicosanoids, the biosynthesis of cholesterol and bile acids, steroid synthesis and metabolism, the synthesis and degradation of vitamin D and retinoic acids, biogenic amines and presumably other morphogens (Nebert and Dalton, 2006). In some cases, the enzymes may activate exogenous compounds to toxins or carcinogens (Androutsopoulos *et al.*, 2009). There may be still a number of CYP enzymes with unknown functions. In human genome, there are 57 genes involved in the synthesis of 57 cytochrome P450 enzymes which are classified into 18 families (Nebert and Russel, 2002). Among them, 50 are microsomal and 7 are mitochondrial enzymes. The tertiary structures of 11 microsomal enzymes are known (Ghosh *et al.*, 2009; Mast *et al.*, 2008; Rowland *et al.*, 2006; Sansen *et al.*, 2007; Schoch *et al.*, 2008; Strushkevich *et al.*, 2008, 2010; Williams *et al.*, 2000, 2003, 2004; Yano *et al.*, 2005), including CYP2R1 which is the major 25-hydroxylase found in human to generate 25(OH)D3 from vitamin D3 (Cheng *et al.*, 2003, 2004). CYP24A1 and CYP11A1 are the only two mitochondrial CYP enzymes whose crystal structures have been reported (Annalora *et al.*, 2010; Mast *et al.*, 2011). The importance of CYP enzymes in human is clearly demonstrated with serious health problems when any mutations in the *CYP* genes occur. One example is that mutations in *CYP*17A1 will lead to mineralcorticoid excess syndromes, glucocorticoid and sex hormone deficiencies, increased risk of prostate cancer and benign prostatic hypertrophy (Nebert and Russel, 2002). In the case of vitamin D metabolism, mutations in *CYP2R1*, responsible for the synthesis of 25(OH)D from vitamin D, and *CYP27B1*, responsible for the 1α-hydroxylation of 25(OH)D to produce the active form 1α,25$(OH)_2$D, will cause vitamin D deficiency-induced rickets and vitamin D-dependent rickets type 1, respectively (Cheng *et al.*, 2003, 2004; St-Arnaud *et al.*, 1997). On the other hand, mutations in *CYP24A1* have been shown recently to induce

idiopathic infantile hypercalcemia, because CYP24A1 is responsible for the degradation of 25(OH)D, the circulating form of vitamin D, and 1α,25$(OH)_2$D, the active form (Schlingmann *et al.*, 2011) (Table 23.1).

23.3 Vitamin D metabolism

Vitamin D was discovered in 1922 as the active anti-rachitic principle found in cod liver oil (McCollum *et al.*, 1922). Until 1964, it was generally believed that either vitamin D2 or vitamin D3 was active without any further metabolism. DeLuca and colleagues first questioned this assumption and provided evidence suggesting that vitamin D3 was metabolized to more polar metabolites which were the active forms responsible for stimulating calcium transport (Norman *et al.*, 1964). During the following several years, intensive efforts carried out by DeLuca and his associates led to the isolation and identification of the first metabolite of vitamin D3, 25(OH)D3 (Blunt *et al.*, 1968). The site of 25(OH)D3 synthesis was soon demonstrated in the rat liver *in vivo* by Ponchon and DeLuca (1969). This finding was subsequently confirmed by Horsting and DeLuca (1969) in their *in vitro* experiments using rat liver homogenates. In the same year, Lawson *et al.* (1969a,b) described a new vitamin D3 metabolite with a loss of hydrogen at C-1 position isolated from chick intestinal nuclei, which was followed by the identification of a unique biological active vitamin D metabolite synthesized in the kidneys (Fraser and Kodicek, 1970). This metabolite was found to be more polar and much more potent than 25(OH)D3 in raising serum calcium and in stimulating calcium transport in the gut. The structure of this active

Table 23.1. Functions and substrates of four major vitamin D CYP enzymes, and disorders resulting from their mutation.

Gene	Enzyme location	Enzyme function	Enzyme substrate	Disorder due to gene mutation
CYP2R1	Microsomal	C-25 hydroxylation	Vitamin D3 Vitamin D2	Vitamin D-dependent Ricket type 1
CYP27A1	Microsomal	C-25 hydroxylation	Vitamin D3	Cerebrotendinous Xanthomatosis
CYP27B1	Mitochondrial	C-1 hydroxylation	25(OH)D3 25(OH)D2	Vitamin D-dependent Ricket type 1
CYP24A1	Mitochondrial	C-24 hydroxylation	25(OH)D3 25(OH)D2[1] 1α,25$(OH)_2$D3 1α,25$(OH)_2$D2	Idiopatathic infantile hypercalcemia

[1] Although no study has been reported, a metabolic pathway similar to that of 1α,25$(OH)_2$D2 C-24 hydroxylation can be assumed.

metabolite was soon identified as 1α,25-dihydroxy-cholecalciferol or 1α,25$(OH)_2$D3 (Holick *et al.*, 1971; Lawson *et al.*, 1971; Norman *et al.*, 1971), and chemically synthesized (Semmler *et al.*, 1972). One additional dihydroxylated metabolite of vitamin D was also found in the blood of pig injected with large amounts of vitamin D3. This new metabolite was first thought to be 21,25-dihydroxycholecalciferol or 21,25$(OH)_2$D3 (Suda *et al.*, 1970), but later was revised as 24,25-dihydroxycholecalciferol or 24,25$(OH)_2$D3 (Holick *et al.*, 1972). A reciprocal synthesis of 24,25$(OH)_2$D3 and 1α,25$(OH)_2$D3 was observed after the infusion of PTH into the thyroid/parathyroidectomized rats, suggesting an inversed regulation of the two enzymes, CYP27B1 and CYP24A1, responsible for the synthesis of these two dihydroxylated metabolites (Garabedian *et al.*, 1972).

At the present time, there are at least 6 naturally occurring CYP enzymes which have been implicated in the hydroxylation of vitamin D to produce 25(OH)D (Ohyama and Yamasaki, 2004). They are CYP2C11, CYP27A1, CYP3A4, CYP2J3, CYP2D25 and CYP2R1. The inclusion of CYP2R1 as a 25-OHase did not occur until 2003 when Cheng *et al.* (2003) screened a cDNA library made from hepatic mRNA of mice deficient in gene encoding the mitochondrial CYP27A1 using a VDR-based ligand activation assay. They soon confirmed that a mutation of *CYP2R1* gene in an individual caused vitamin D deficient rickets (Cheng *et al.*, 2004). Using a cell-free re-constituted enzyme system, Sakaki and co-workers demonstrated that CYP2R1 was able to hydroxylate either vitamin D3 or vitamin D2 to their corresponding 25-hydroxylated metabolites (Shinkyo *et al.*, 2004). However, this is not the case for CYP27A1which can only hydroxylate vitamin D3 to 25(OH)D3, not vitamin D2 to 25(OH)D2. Instead, this enzyme produces C-24 or C-27 hydroxylated vitamin D2 metabolite. So far, CYP27B1 is the only enzyme involved in the 1α-hydroxylation of vitamin D, and CYP24A1 is the primary one to hydroxylate 25(OH)D and 1α,25$(OH)_2$D at C24 position (Figure 23.1). In this regard, Sakaki *et al.* have demonstrated that CYP24A1 converts 1α,25$(OH)_2$D3 to calcitroic acid by a six-step monooxygenation including C-24 hydroxylation as the first step of 1 α,25$(OH)_2$D3 catabolism (Sakaki *et al.*, 1999). A similar CYP24A1-dependent C-24 hydroxylation of 1 α,25$(OH)_2$D2 has been observed as the first step of the 1 α,25$(OH)_2$D2 degradative pathway (Urushino *et al.*, 2009).

23.4 Study of vitamin D-25-hydroxylases in prostate cells (CYP2R1)

The demonstration of the presence of 25-OHase in prostate cells was accomplished by (1) three functional assays, (2) direct determination of the presence of 1α,25$(OH)_2$D3 after the addition of vitamin D3 into prostate cells, and (3) the expression of *CYP2R1* and *CYP27A1* mRNA in prostate cells (Flanagan *et al.*, 2006).

In the functional assays, the expression of *CYP24A1* and insulin-like growth factor binding protein 3 genes were used. These two genes are highly responsive to the stimulation by 1α,25$(OH)_2$D3. Since the prostate cells in cultures were treated with vitamin D3, the effects on these two genes could only occur when 25(OH)D3 was first synthesized from vitamin D3 through 25-OHase catalysis prior to the synthesis of 1α,25$(OH)_2$D3. Using this approach, it was

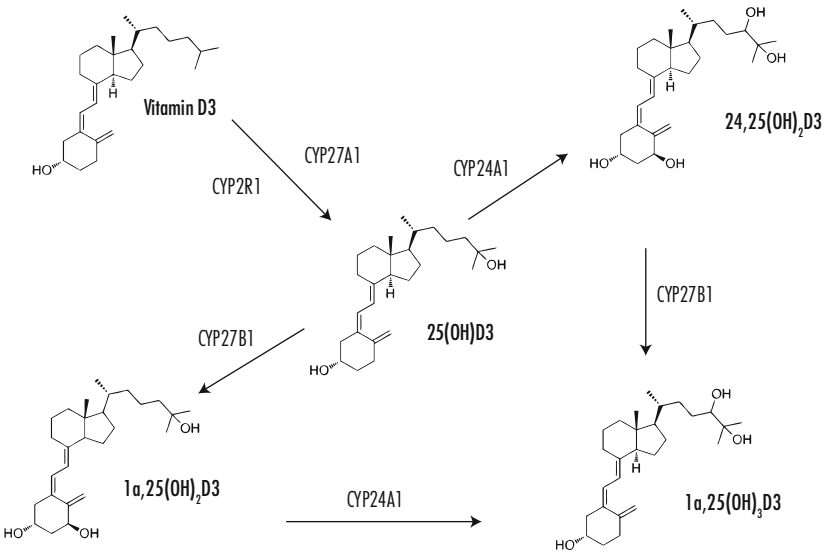

Figure 23.1. Metabolism of vitamin D3 involving different CYP enzymes.

shown that vitamin D3 caused a dose-dependent up-regulation of these two genes in PZ-HPV-7 prostate cells (Flanagan *et al.*, 2006). In the third functional assay to demonstrate the presence of 25-OHase activity, Flanagan *et al.* (2006) studied the ability of vitamin D3 to inhibit (^{3}H)-thymidine incorporation into prostate cell as an index of anti-proliferation. They showed that (^{3}H)-thymidine incorporation into DNA was inhibited ~40%, when PZHPV-7 prostate cells were exposed to 10^{-6} M of vitamin D3 for 18 hours, as compared to ~80% inhibition with 10^{-7} M of 1α,25(OH)$_2$D3 for 18 hours. Although no 25(OH)D3 could be detected by protein binding assay (Chen *et al.*, 1990a) after incubating PZHPV-7 cells with vitamin D3 for 24 hours, the presence of 1α,25(OH)$_2$D3 in the cultures was detected by a very sensitive and specific thymus receptor binding assay (Chen *et al.*, 1990b).

In order to determine which 25-OHase is the predominate form in prostate cells, the expression of three human-related 25-OHases, *CPY2R1*, *CYP27A1*, and *CYP3A4*, was examined in human normal prostate and liver tissues, and in prostate cancer cell lines. It was found that *CYP2R1* was expressed in a much greater extent in prostate tissue and cell lines than in liver tissue. Very little *CYP27A1* or *CYP3A4* was expressed in normal prostate tissue and cell lines, whereas both *CYP* genes were highly expressed in liver tissue. Therefore, the results suggest that CYP2R1 is more likely the 25-OHase responsible for the hydroxylation of vitamin D3 to 25(OH)D3 in the prostate (Flanagan *et al.*, 2006). Very little is known about the regulation of *CYP2R1*, except that the enzyme can be down-regulated by 1α,25(OH)$_2$D3 (Ellfolk *et al.*, 2009), suggesting that the promoter of *CYP2R1* gene may have negative VDRE(s).

23.5 Study of CYP27B1 in prostate cells

The location of *CYP27B1* gene was mapped to chromosome 12q13-14 (Miller and Portale, 2000). The cDNAs encoding the mouse, rat and human CYP27B1 have been cloned independently by four research groups (Fu *et al.*, 1997; Shinke *et al.*, 1997; St-Arnaud *et al.*, 1997; Takeyama *et al.*, 1997). The cloned human *CYP27B1* gene consisting of 9 exons and 8 introns has 1,524 base pairs encoding 508 amino acids with a molecular weight of 58 kDa. The promoter of mouse and human *CYP27B1* genes have also been cloned with a length of 1.7 kb and 1.4 kb, respectively (Brenza *et al.*, 1998; Kong *et al.*, 1999).

23.5.1 Differential expression of CYP27B1 activity in prostate cells

CYP27B1 is expressed in certain prostate cells as analyzed by their ability to convert ^{3}H-25(OH) D3 to ^{3}H-1α,25(OH)$_2$D3 in cultures (Schwartz *et al.*, 1998), by mRNA expression using real-time qPCR (Chen and Holick, 2001), and by promoter/luciferase assay (Wang *et al.*, 2003). The expression suggests that local production of 1α,25(OH)$_2$D3 could provide an important cell growth regulatory mechanism in prostate cells (Chen *et al.*, 2003; Wang *et al.*, 2003). However, there is a differential expression of CYP27B1 activity among the primary cultures of prostate cells derived from cancerous, benign prostatic hypertrophy and normal tissue, as well as among prostate cell lines either derived from normal prostate tissue (PZHPV-7 cells) or from prostate cancer cells (PC-3, DU145 and LNCaP cells) (Table 23.2). Similar observations were reported by Hsu *et al.* (2001). No or very little CYP27B1 activity and mRNA expression were found in LNCaP cells, that supports the data showing that LNCaP cells were not responsive to the addition of 25(OH)D3 (Skowronski *et al.*, 1995; Whitlatch *et al.*, 2002), because the cells could not convert 25(OH)D3 to 1α,25(OH)$_2$D3. Therefore, by transfecting LNCaP cells with *CYP27B1* cDNA, Whitlatch *et al.* (2002) were able to restore their responsiveness to 25(OH)D3.

Table 23.2. Comparison of the enzymatic activity, mRNA level and gene promoter activity of *CYP27B1* in prostate cell lines.

	Enzyme activity (% conversion)	mRNA level[1] (fold)	Promoter luciferase activity	
			AN2	AN5
LNCaP	ND[2]	1	95±18	ND
PC-3	1.5±0.8	5	1,633±118	5,364±290
DU145	3.7±0.6	51	8,581±223	14,432±863
PZ-HPV-7	37±3.0	157	30,076±857	46,347±735

[1] Real-time PCR.

[2] Non-detectable.

23.5.2 Regulation of prostate CYP27B1 activity

Unlike the enzyme in renal cells (Chen *et al.*, 1989), the promoter activity of *CYP27B1* and the enzymatic activity of CYP27B1 are not regulated by PTH or calcium in PZHPV-7 prostate cells (Young *et al.*, 2004). However, the enzyme is down-regulated by its own product, 1α,25$(OH)_2$D3, at the promoter and enzyme activity levels. Moreover, it has been shown that EGF up-regulates *CYP27B1* at both the transcriptional and translational levels as evident from the luciferase promoter assay, real-time quantitative RT-PCR analysis and enzyme activity measurement using high performance liquid chromatography (Wang *et al.*, 2004). The EGF-dependent up-regulation of the promoter activity is inhibited by MAPKK inhibitor, PD98059 (Wang *et al.*, 2004), suggesting that MAPK signaling pathway may be one mechanism involved in the regulation of prostate *CYP27B1* gene expression by EGF to stimulate 1α,25$(OH)_2$D3 synthesis that in turn inhibits cell proliferation as a feedback regulator of cell growth. Preliminary data using Chip assay indicate that EGF/EGFR complex may directly bind to the promoter of *CYP27B1* in PZ-HPV7 cells. The direct binding of EGF/EGFR complex to promoters and to act as a transcriptional factor to regulate cell growth has been demonstrated previously (Lin *et al.*, 2001). However, EGF has no effect on *CYP27B1* promoter activity in LNCaP cells (Wang *et al.*, 2004), suggesting that the ability of EGF to stimulate 1α,25$(OH)_2$D3 synthesis may be abolished or diminished in cancer cells. Overall, the data suggest that EGF may play an important role in the development of prostate cancer (Figure 23.2), and CYP27B1 is likely a tumor suppressor in the prostate (Chen, 2008). *CYP27B1* expression can also be up-regulated by suberoylanilide hydroxamic acid, an inhibitor of histone deacetylases (Wang *et al.*, 2008). Since the inhibitors of histone deacetylases have been shown to cause growth arrest, and promote differentiation and apoptosis of many tumor cells by altering the transcription of genes (Xu *et al.*, 2007), it is likely that *CYP27B1* gene may be a new target for suberoylanilide hydroxamic acid through its action on the acetylation status of the histone tails of chromatin.

In glioblastoma TX3868 cells (Maas *et al.*, 2001), cultured human keratinocytes (Flanagan *et al.*, 2003), and HKC-8 human proximal tubular cells (Wu *et al.*, 2007), expression of *CYP27B1* splice variants has been reported as one mechanism to regulate CYP27B1 activity in a tissue-specific manner. In addition, trafficking of the CYP27B1 substrate 25(OH)D and CYP27B1 enzyme itself may regulate the synthesis of 1α,25$(OH)_2$D as a mean to modulate 1α,25$(OH)_2$D action as shown in the human innate immune response (Adams *et al.*, 2007).

23.6 Study of 24-hydroxylation by CYP24A1 in prostate cells

CYP24A1 is responsible for the hydroxylation at C-24 of 25(OH)D and 1α,25$(OH)_2$D through a six-step monooxygenation pathway (Sakaki *et al.*, 1999), that serves as the initial step to inactivate 1α,25$(OH)_2$D, and is a major mechanism to terminate the actions of this active hormone inside the cells. The final water soluble calcitroic acid which is likely further metabolized is excreted into the urine (Schuster, 2011). Because 1α,25$(OH)_2$D is synthesized in the mitochondria, acts through binding to VDR in the nucleus, and then is degraded by mitochondrial CYP24A1 within

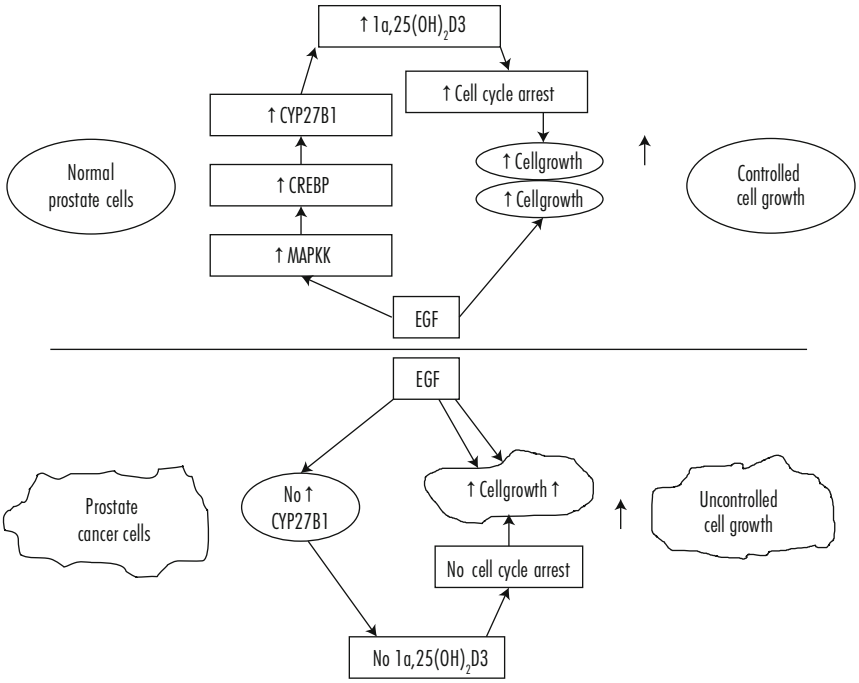

Figure 23.2. Interactions among EGF, CYP27B1 and prostate cell growth. An up-regulation of CYP27B1 by EGF is hypothesized to be responsible for the normal growth of prostate cells (upper panel), whereas dysregulation of CYP27B1 by EGF may cause uncontrollable prostate cell growth (lower panel). CREBP = cAMP-responsive element binding protein; EGF = epidermal growth factor; MAPKK = mitogen activated protein kinase kinase.

the cells and has little chance to be released into the circulation, no $1\alpha,25(OH)_2D$ has been detected in the circulation of nephrectomized animals (Shultz *et al.*, 1983; Reeve *et al.*, 1983).

23.6.1 Expression and the regulation of CYP24A1 in prostate cancer cells

Like many other cells, prostate cells are known to express *CYP24A1* (Flanangan *et al.*, 2009; Ly *et al.*, 1999; Miller *et al.*, 1995; Skowronski *et al.*, 1993). The gene is one of the target genes of $1\alpha,25(OH)_2D$ and is up-regulated by $1\alpha,25(OH)_2D$ and down-regulated by PTH (Garabedian *et al.*, 1972). Therefore, it has been used as a biomarker for the synthesis of $1\alpha,25(OH)_2D3$ from 25(OH)D3 (Flanagan *et al.*, 2006). Elevated *CYP24A1* expression has been reported in several types of human cancer (Cross *et al.*, 2005; Friedrich *et al.*, 2003) and prostate cancer cell line, DU-145 (Ly *et al.*, 1999). This is in line with a quantitative mapping of amplicon structure by an array comparative genomic hybridization technique which identified CYP24A1 as a candidate oncogene whose overexpression is likely to lead to abrogation of growth control mediated by vitamin D (Albertson *et al.*, 2000). Treatment of normal prostate PNT-2 cells with the methylation inhibitor 5-aza-2'-deoxycytidine together with the deacetylation inhibitor trichostatin A has been shown to elevate both CYP27B1 and *CYP24A1* mRNA expression demonstrating that the expression of

these two vitamin D hydroxylases are likely under epigenetic control (Khorchide *et al.*, 2005). More recently, an inversed correlation between *CYP24A1* expression and methylation in the promoter region of *CYP24A1* gene in prostate cancer cell lines has been observed, suggesting an epigenetic regulation of *CYP24A1* (Luo *et al.*, 2010). Deeb *et al.* (2011) studied methylation of the *CYP24A1* gene promoter in endothelial cells isolated from the cancerous and benign prostate tissues and found that the average *CYP24A1* promoter methylation was 20% from the tumor environment compared with 8.2% in the benign microenvironment. Whether the epigenetic regulation of CYP24A1 has any implications on the prostate tumor progression is unknown at the present time and remains to be determined.

23.6.2 CYP24A1-resistant vitamin D analogs

Since 1α,25$(OH)_2$D is subject to CYP24A1 degradation, any of its analogs which are resistant to CYP24A1 hydroxylation will have longer half-life and potentially could be more active. Several structural modifications of the 1α,25$(OH)_2$D3 molecule have been accomplished to achieve this goal. For example, ED-71, a widely-studied 1α,25$(OH)_2$D3 analog with an addition of 3-hydroxypropoxy group attached in β-configuration to the C-2 position of the 1α,25$(OH)_2$D3 molecule, is a poor substrate for CYP24A1 (Dr. Noboru Kubodera, personal communication). Likewise, O2C3, the C-2 epimer of ED-71, is also resistant to CYP24A1 hydroxylation (Abe *et al.*, 2005). A list of 19-nor-1α,25$(OH)_2$D3 analogs with a modification at the C-2 of this molecule have been studied for their anti-proliferative and pro-differentiation activities (Chen *et al.*, 2007; Kittaka *et al.*, 2007; Ono *et al.*, 2003). One of these compounds, MART-10, has been shown to be 500-1000 times more active in inhibiting prostate cell proliferation and about 300-500 times less susceptible to CYP24A1 degradation than 1α,25$(OH)_2$D3 (Chen *et al.*, 2007; Flanagan *et al.*, 2009). To study the docking of MART-10 into CYP24A1, a human CYP24A1 substrate binding site based on the published crystal structure of rat CYP24A1 (Annalora *et al.*, 2010) was generated (Iglesias-Gato *et al.*, 2011). According to this model, A-ring of MART-10 is positioned over heme group and the 3-hydroxypropyl group on the A-ring is located on the groove of I-helix kink forming hydrogen bonds with the back bone of L325 and E329 and blocking the groove. Consequently, the side-chain of MART-10 is far away from the heme group and is unavailable for 24-hydroxylation, suggesting that MART-10 will have longer half-life in the prostate. Thus, the results further suggest that analogs with modification at C-2 position, such as MART-10, could be developed for the treatment of prostate cancer due to its longer bioavailability and greater potency (Chen *et al.*, 2007).

23.6.3 Inhibitors of CYP24A1

An alternative approach to enhance the half-life and activity of 1α,25$(OH)_2$D is to use the inhibitors of CYP24A1 to decrease the degradation of the active hormone by 24-hydroxylation. A range of P450 inhibitors are currently available, such as Ketoconazole and Liarazole (Figure 23.3). These azole compounds work directly by binding to the heme iron through the azole nitrogen. Therefore, they are relatively non-selective. More selective CYP24 A1 inhibitors have been developed by Novartis, such as VID 400, and Cytochroma Inc., CTA018 (Posner *et al.*,

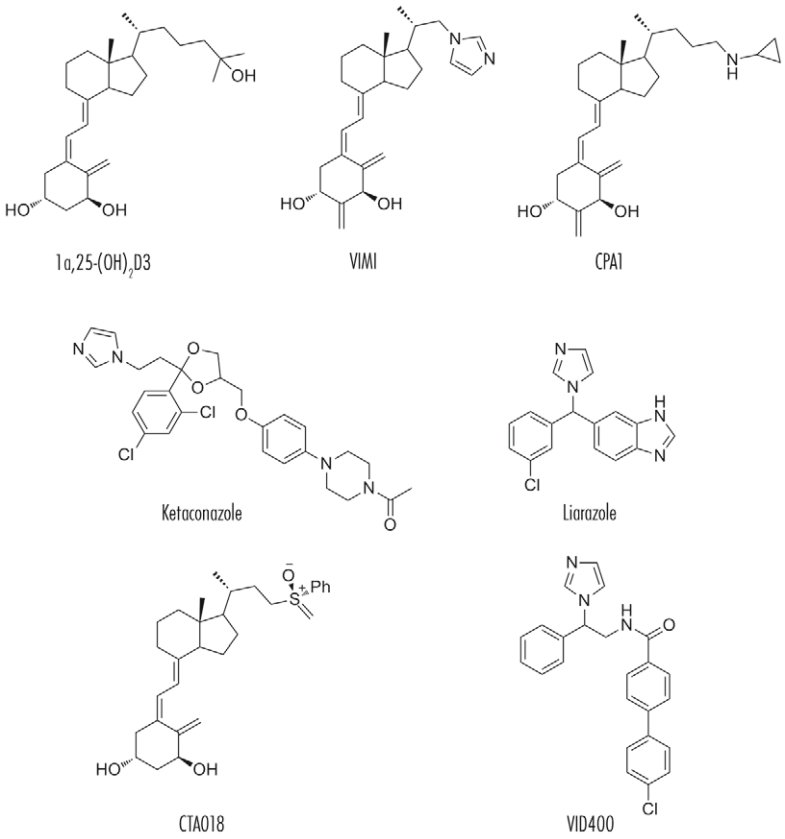

Figure 23.3. Chemical structures of certain specific and non-specific CYP24A1 inhibitors.

2004). CTA018 has the ring structure of 1α,25(OH)$_2$D3, but with a sulfoximine functional group replacing C-24. A phase II clinical trial using CTA018 is underway for topical treatment of mild to moderate psoriasis. Recently, two new vitamin D-like CYP24A1 inhibitors, VIMI and CPA1, have been developed (Chiellini *et al.*, 2012). CPA1 shows an 80-1 selective inhibition of CYP24A1 over CYP27B1.

23.7 25(OH)D can be active without 1α-hydroxylation

After the discovery of 25(OH)D3 in 1968, Olson and DeLuca reported that 25(OH)D3 added into the incubation media was able to enhance calcium absorption from the lumen in studies using an isolated small intestine loop. From this finding, they concluded that 25(OH)D3 was the metabolic active form of vitamin D3 and had direct effect on calcium transport (Olson and DeLuca, 1969). With the discovery of 1α,25(OH)$_2$D, this 25(OH)D effect was believed to be simply a pharmacological effect. Three decades later, the direct effect of 25(OH)D3 on PTH secretion was observed by Ritter *et al.* (2006), who used cytochrome p450 inhibitor clotrimazole

to block the conversion of 25(OH)D3 to 1α,25$(OH)_2$D3 in the bovine parathyroid cells. The direct effect caused by 25(OH)D3 in the bovine parathyroid cells was subsequently shown to be VDR dependent (Ritter and Brown, 2011). Similarly, Lou *et al.* demonstrated the antiproliferative action of 25(OH)D3 on human MCF-7 breast cancer cells and in the primary cultures of kidney, skin and prostate cells prepared from *Cyp27b1* knockout mice (Lou *et al.*, 2010). The authors demonstrated that the action induced by 25(OH)D3 was mediated by VDR, and 25(OH)D3 had identical binding mode as 1α,25$(OH)_2$D3 in these cells. More importantly, a synergistic effect of 25(OH)D3 with 1α,25$(OH)_2$D3 in *Cyp27b1*$^{-/-}$ cells was observed. The authors suggest that a synergism between 25(OH)D3 and 1α,25$(OH)_2$D3 might be physiologically important. More recently, DeLuca *et al.* (2011) used the VDR knockout model to evaluate the hypercalcemic toxicity induced by high doses of vitamin D3 and 25(OH)D3 and demonstrated that high concentrations of 25(OH)D3 could bind to VDR and could induce gene transcription in the *Cyp27B1*$^{-/-}$ mice. Since no 1α,25$(OH)_2$D3 was detected in the serum of these *Cyp27B1*$^{-/-}$ mice, they concluded that 25(OH)D3, not 1α,25$(OH)_2$D3, was likely responsible for the toxicity of vitamin D excess. Using different approach, Munetsuna *et al.* (2011) also demonstrated that 1α-hydroxylation of 25-hydroxy-19-nor-vitamin D3 was not required for its biological activity in PZHPV-7 prostate cells.

23.8 Summary and conclusions

This review focuses on the findings obtained during the past decade regarding the expression of the three cytochrome P450 enzymes, CYP27B1, CYP2R1, and CYP24A1, and their roles in the activation and inactivation of vitamin D3 in prostate cells. In summary, prostate cells have all these enzymes and are capable of synthesizing vitamin D3 to 25(OH)D3 and then to 1α,25$(OH)_2$D3 (Figure 23.4). Furthermore, 25(OH)D3 can act through VDR without converting to 1α,25$(OH)_2$D3. The autocrine synthesis of 25(OH)D3 and 1α,25$(OH)_2$D3 in prostate cells may imply that vitamin D plays important role in maintaining the normal functions of prostate cells through a vast number of genes it can regulate and the versatile actions it can cause (Figure 23.4). Although very little is known about the regulation of CYP2R1 and how vitamin D3 and 25(OH)D3 are delivered into the prostate, we can speculate that limited trafficking of vitamin D3 and 25(OH)D3 into the prostate may occur in certain pathological conditions, and may interfere with the synthesis of 25(OH)D3 and 1α,25$(OH)_2$D3 and therefore affecting their actions in the prostate similar to that shown in the human innate immune response (Adams *et al.*, 2007).

Thus, we need to develop analogs of vitamin D3, 25(OH)D3 and 1α,25$(OH)_2$D3 which are not only less calcemic and more resistant to CYP24A1 hydroxylation, such as MART-10, but also can be easily taking up by the cells to bind to VDR directly or to be activated before exerting their biological actions.

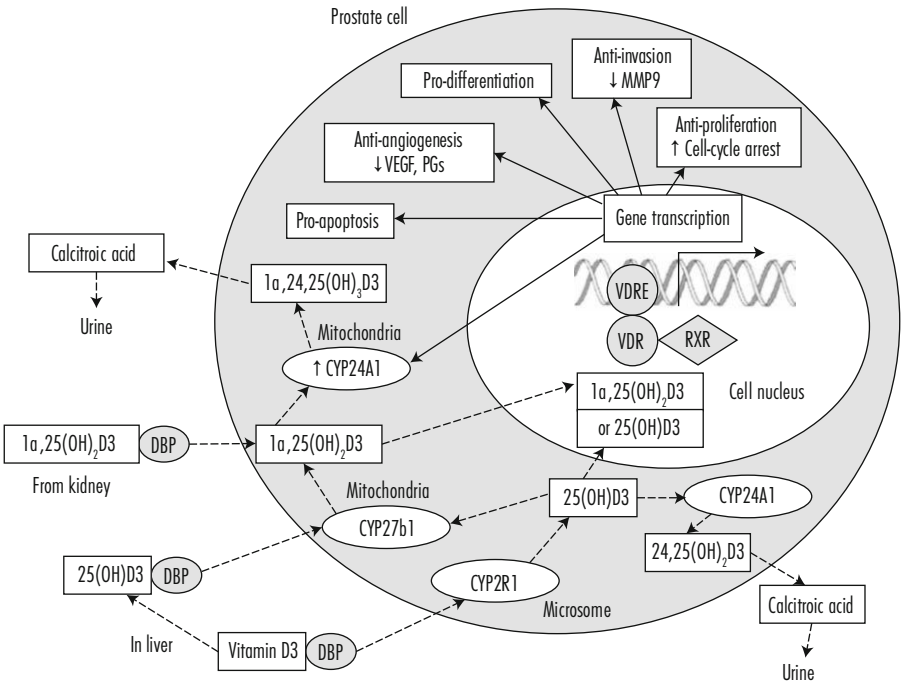

Figure 23.4. Metabolism and functions of vitamin D3 in prostate cells. DBP = vitamin D binding protein; PGs = prostaglandins; RXR = retinoid X receptor; VDR = vitamin D receptor; VDRE = vitamin D response element; VGEF = vascular endothelial growth factor.

References

Abe, D., Sakaki, T., Kusudo, T., Kittaka, A., Saito, N., Suhara, Y., Fujishima, T., Takayama, H., Hamamoto, H., Kamakura, M., Ohta, M. and Inouye, K., 2005. Metabolism of 2 α-propoxy-1α,25-dihydroxyvitamin D3 and 2α-(3-hydroxypropoxy)-1α,25-dihydroxyvitamin D3 by human CYP27A1 and CYP24A1. Drug Metab Dispos 33, 778-784.

Adams, J.S., Chen, H., Chun, R., Ren, S., Wu, S., Gacad, M., Nguyen, L., Ride, J., Liu, P., Modlin, R. and Hewison, M., 2007. Substrate and enzyme trafficking as a means of regulating 1,25-dihydroxyvitamin D synthesis and action: the human innate immune response. J Bone Miner Res 22 Suppl 2,V20-V24.

Albertson, D.G., Ylstra, B., Segraves, R., Collins, C., Dairkee, S.H., Kowbel, D., Kuo, W.L., Gray, J.W. and Pinkel, D., 2000. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nat Genet 25, 144-146.

Androutsopoulos, V.P., Tsatsakis, A.M. and Spandidos, D.A., 2009. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer 9, 187-203.

Annalora, A.J., Goodin, D.B., Hong, W.X., Zhang, Q., Johnson, E.F. and Stout, C.D., 2010. Crystal structure of CYP24A1, a mitochondrial cytochrome P450 involved in vitamin D metabolism. J Mol Biol 396, 441-451.

Blunt, J.W., Tanaka, Y. and DeLuca, H.F., 1968. The biological activity of 25-hydroxy cholecalciferol, a metabolite of vitamin D3. Proc Natl Acad Sci USA 61, 717-718.

Brenza, H.L., Kimmel-Jehan, C., Jehan, F., Shinki, T., Wakino, S., Anazawa, H., Suda, T. and DeLuca, H.F., 1998. Parathyroid hormone activation of the 25-hydroxyvitamin D3-1alpha-hydroxylase gene promoter. Proc Natl Acad Sci U S A 95, 1387-1391.

Chen, T.C., Curthoys, N.P., Lagenaur, C. and Puschett, J.B., 1989. Characterization of primary cell cultures derived from rat renal proximal tubules. In vitro 25, 714-722.

Chen, T.C., Turner, A.K, and Holick, M.F., 1990a. Methods for the determination of the circulating concentration of 25-hydroxyvitamin D. J Nutr Biochem 1, 315-319.

Chen, T.C., Turner, A.K. and Holick, M.F., 1990b. A method for the determination of the circulating concentration of 1,25-dihydroxyvitamin D. J Nutr Biochem 1, 320-327.

Chen, T.C., Wang, L., Whitlatch, L.W., Flanagan, J.N. and Holick, M.F., 2003. Prostatic 25-hydroxyvitamin D-1α-hydroxylase and its implication in prostate cancer. J Cellular Biochem 88, 315-322.

Chen, T.C., Persons, K.S., Zheng, S., Mathieu, J., Holick, M.F., Lee, Y.F., Bao, B., Arai, M.A. and Kittaka, A., 2007. Evaluation of C-2-substituted 19-nor-1α,25-dihydroxyvitamin D3 analogs as therapeutic agents for prostate cancer. J Steroid Biochem Mol Biol 103, 717-720.

Chen, T.C., 2008. 25-hydroxyvitamin D-1 alpha-hydroxylase (CYP27B1) is a new class of tumor suppressor in the prostate. Anticancer Research 28 (4A), 2015-2018.

Chen, T. C. and Holick, M. F., 2003. Vitamin D and prostate cancer prevention and treatment. Trends Endocrinol Metab 144, 423-430.

Cheng, J.B., Motola, D.L., Mangelsdorf, D.J. and Russell, D.W., 2003. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxilase. J Biol Chem 278, 38084-38093.

Cheng, J.B., Levine, M.A., Bell, N.H., Mangelsdorf, D.J. and Russell, D.W., 2004. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 101, 7711-7715.

Chiellini, G., Rapposelli, S., Zhu, J., Massarelli, I., Saraceno, M., Bianucci, A.M., Plum, L.A., Clagett-Dame, M., and DeLuca, H.F., 2012. Synthesis and biological activities of vitamin D-like inhibitors of CYP24 hydroxylase. Steroids 77, 212-223.

Cross, H.S., Bises, G., Lechner, D., Manhardt, T. and Kallay, E., 2005. The vitamin D endocrine system of the gut-its possible role in colorectal cancer prevention. J Steroid Biochem Mol Bio 97, 121-128.

Deeb, K.K., Luo, W., Karpf, A.R., Omilian, A.R., Bshara, W., Tian, L., Tangrea, M.A., Morrison, C.D., Johnson, C.S. and Trump, D.L., 2011. Differential vitamin D 24-hydroxylase/CYP24A1 gene promoter methylation in endothelium from benign and malignant human prostate. Epigenetics 6, 994-1000.

DeLuca, H.F., 1976. Metabolism of vitamin D: current status. Am J Clin Nutr 29, 1258-1270.

DeLuca, H.F., Prahl, J.M. and Plum, L.A., 2011. 1,25-Dihydroxyvitamin D is not responsible for toxicity caused by vitamin D or 25-hydroxyvitamin D. Arch Biochem Biophys 505, 226-230.

Ellfolk, M., Norlin, M., Gyllensten, K., and Wikvall, K., 2009. Regulation of Human Vitamin D3 25-Hydroxylases in Dermal Fibroblasts and Prostate Cancer LNCaP Cells. Mol Pharmacol 75, 1392-1399.

Flanagan, J.N., Young, M.V., Persons, K.S., Wang, L., Whitlatch, L.W., Holick, M.F. and Chen, T.C., 2006. Human prostate cells can synthesize 1α,25-dihydroxyvitamin D from vitamin D: Implication for prostate cancer chemoprevention by vitamin D. Anticancer Res 26, 2567-2572.

Flanagan, J.N., Wang, L., Tangpricha, V., Reichrath, J., Chen, T.C. and Holick, M.F., 2003. Regulation of the 25-hydroxyvitamin D-1alpha-hydroxylase gene and its splice variant. Recent Results Cancer Res 164, 157-167.

Flanagan, J.N., Zheng, S., Chiang, K.C., Kittaka, A., Sakaki, T., Nakabayashi, S., Zhao, X., Spanjaard, R.A., Persons, K.S., Mathieu, J.S., Holick, M.F. and Chen, T.C., 2009. Evaluation of 19-nor-2α-(3-hydroxypropyl)-1α,25-dihydroxyvitamin D3 As a Therapeutic Agent for Androgen-dependent Prostate Cancer. Anticancer Research 29, 3547-3554.

Fraser, D.R. and Kodicek, E., 1970. Unique biosynthesis by kidney of a biological active vitamin D metabolite. Nature 228, 764-766.

Friedrich, M., Rafi, L., Mitschele, T.,Tilgen, W., Schmidt W. and Reichrath, J., 2003. Analysis of the vitamin D system in cervical carcinomas, breast cancer and ovarian cancer. Recent results cancer Res 164, 239-246.

Fu, G.K., Lin, D., Zhang, M.Y., Bikle, D.D., Shackleton, C.H., Miller, W.L. and Portale, A.A., 1997. Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. Mol Endocrinol 11, 1961-1970.

Garabedian, M., Holick, M.F., Deluca, H.F. and Boyle, I.T., 1972. Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. Proc Natl Acad Sci USA 69, 1673-1676.

Ghosh, D., Ghosh, D., Griswold, J., Erman, M. and Pangborn, W., 2009. Structural basis for androgen specificity and oestrogen synthesis in human aromatase. Nature 457, 219-223.

Grant, W.B., 2012. Ecological studies of the UVB-vitamin D-cancer hypothesis. Anticancer Res 32, 223-236.

Holick, M.F., MacLaughlin, J.A., Clark, M.B., Holick, S.A., Potts, J.T. Jr, Anderson, R.R., Blank, I.H., Parrish, J.A. and Elias, P., 1980. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science 210, 203-205.

Holick, M.F., Schnoes, H.K., DeLuca, H.F., Gray, R.W., Boyle, I.T. and Suda, T., 1972. Isolation and identification of 24,25-dihydroxycholecalciferol, a metabolite of vitamin D made in the kidney. Biochemistry 11, 4251-4255.

Holick, M.F., Schnoes, H.K. and DeLuca, H.F., 1971. Identification of 1,25-dihydroxycholecalciferol, a form of vitamin D3 metabolically active in the intestine. Proc Natl Acad Sci USA 68, 803-804.

Horsting, M. and DeLuca, H.F., 1969. *In vitro* production of 25-hydroxycholecalciferol. Biochem Biophys Res Commun 36, 251-256.

Hsu, J.Y., Feldman, D., McNeal, J.E. and Peehl, D.M., 2001. Reduced 1alpha-hydroxylase activity in human prostate cancer cells correlates with decreased susceptibility to 25-hydroxyvitamin D3-induced growth inhibition. Cancer Res 61, 2852-2856.

Iglesias-Gato, D., Zheng, S., Flanagan, J.N., Jiang, L., Kittaka, A., Sakaki, T., Yamamoto, K., Itoh, T., Lebrasseur, N.K., Norstedt, G. and Chen, T.C., 2011. Substitution at carbon 2 of 19-nor-1α,25-dihydroxyvitamin D(3) with 3-hydroxypropyl group generates an analogue with enhanced chemotherapeutic potency in PC-3 prostate cancer cells. J Steroid Biochem Mol Biol 127, 269-275.

Khorchide, M., Lechner, D. and Cross, H.S., 2005. Epigenetic regulation of vitamin D hydroxylase expression and activity in normal and malignant human prostate cells. J Steroid Biochem Mol Biol 93, 167-172.

Kittaka, A., Saito, N., Honzawa, S., Takenouchi, K., Ishizuka, S., Chen, T.C., Peleg, S., Kato, S. and Arai, M.A., 2007. Creative synthesis of novel vitamin D analogs for health and disease. J Steroid Biochem Mol Biol 103, 269-276.

Kong, X.F., Zhu, X.H., Pei, Y.L., Jackson, D.M. and Holick, M.F., 1999. Molecular cloning, characterization, and promoter analysis of the human 25-hydroxyvitamin D3-1alpha-hydroxylase gene. Proc Natl Acad Sci USA 96, 6988-6993.

Lawson, D.E., Wilson, P.W. and Kodicek, E., 1969a. New vitamin D metabolite localized in intestinal cell nuclei. Nature 222, 171-172.

Lawson, D.E., Wilson, P.W. and Kodicek, E., 1969b. Metabolism of vitamin D. A new cholecalciferol metabolite, involving loss of hydrogen at C-1, in chick intestinal nuclei. Biochem J 115, 269-277.

Lawson, D.E., Fraser, D.R., Kodicek, E., Morris, H.R. and Williams, D.H., 1971. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. Nature 230, 228-230.

Lin, S.Y., Makino, K., Xia, W., Matin, A., Wen, Y., Kwong, K.Y., Bourguignon, L. and Hung, M.C., 2001. Nuclear localization of EGF receptor and its potential new role as a transcription factor. Nat Cell Biol 3, 802-808.

Lou, Y.R., Molnár, F., Peräkylä, M., Qiao, S., Kalueff, A.V., St-Arnaud, R., Carlberg, C. and Tuohimaa, P., 2010. 25-Hydroxyvitamin D(3) is an agonistic vitamin D receptor ligand. J Steroid Biochem Mol Biol 118, 162-170.

Luo, W., Karpf, A.R., Deeb, K.K., Muindi, J.R., Morrison, C.D., Johnson, C.S. and Trump, D.L., 2010. Epigenetic regulation of vitamin D 24-hydroxylase/CYP24A1 in human prostate cancer. Cancer Res 70, 5953-5962.

Ly, H.L., Zha, X.Y., Holloway, L., and Feldman, D., 1999. Liarozole acts synergistically with 1α,25-Dihydroxyvitamin D3 to inhibit growth of DU 145 human prostate cancer cells by blocking 24-hydroxylase activity. Endocrinology 140, 2071-2076.

Maas, R.M., Reus, K., Diesel, B., Steudel, W.I., Feiden, W., Fischer, U. and Meese, E., 2001. Amplification and expression of splice variants of the gene encoding the P450 cytochrome 25-hydroxyvitamin D(3) 1,alpha-hydroxylase (CYP 27B1) in human malignant glioma. Clin Cancer Res 7, 868-875.

Mast, N., Annalora, A.J., Lodowski, D.T., Palczewski, K., Stout, C.D. and Pikuleva, I.A., 2011. Structural basis for three-step sequential catalysis by the cholesterol side chain cleavage enzyme CYP11A1. J Biol Chem 286, 5607-5613.

Mast, N., White, M.A., Bjorkhem, I., Johnson, E.F., Stout, C.D. and Pikuleva, I.A., 2008. Crystal structures of substrate-bound and substrate-free cytochrome P450 46A1, the principal cholesterol hydroxylase in the brain. Proc Natl Acad Sci USA 105, 9546-9551.

McCollum, E. V., Simmonds, N., Becker J.E. and Shipley, P.G., 1922. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J Biol Chem 53, 293-298.

Miller, G.J., 1998. Vitamin D and prostate cancer: biologic interactions and clinical potentials. Cancer Metastasis Rev 17, 353-360.

Miller, G.J., Stapelton, G.E., Hedlund, T.E. and Moffatt, K.A., 1995. Vitamin D receptor expression, 24-hydroxylase activity, and inhibition of growth by 1α,25-dihydroxyvitamin D3 in seven human prostatic carcinoma cell lines. Clin Cancer Res 1, 997-1003.

Miller, W.L. and Portale, A.A., 2000. Vitamin D 1 alpha-hydroxylase. Trends Endocrinol Metab 11, 315-319.

Munetsuna, E., Nakabayashi, S., Kawanami, R., Yasuda, K., Ohta, M., Arai, M.A., Kittaka, A., Chen, T.C., Kamakura, M., Ikushiro, S. and Sakaki, T., 2011. Mechanism of the Anti-proliferative Action of 25-Hydroxy-19-nor-vitamin D3 in Human Prostate Cells. J Mol Endocrinol 47, 209-218.

Nebert, D.W. and Dalton, T.P., 2006. The role of cytochrome P450 enzymes in endogenous signaling pathways and environmental carcinogenesis. Nature Rev/Cancer 6, 947-960.

Nebert, D.W. and Russel, D.W., 2002. Clinical importance of the cytochromes P450. Lancet 360, 1155-1162.

Norman, A.W., Lund, J. and DeLuca, H.F., 1964. Biological Active forms of vitamin D3 in kidney and intestine. Arch Biochem Biophys 108, 12-21.

Norman, A.W., Myrtle, J.F., Midgett, R.J., Nowicki, H.G., Williams, V. and Popják, G., 1971. 1,25-dihydroxycholecalciferol: identification of the proposed active form of vitamin D3 in the intestine. Science 173, 51-54.

Ohyama, Y. and Yamasaki, T., 2004. Eight cytochrome P450s catalyze vitamin D metabolism. Front Biosci 9, 3007-3018.

Olson, E.B. and DeLuca, H.F., 1969. 25-hydroxycholecalciferol: direct effect on calcium transport. Science 165, 405-407.

Ono, K. Yoshida, A, Saito, N., Fujishima, T., Honzawa, S., Suhara, Y., Kishimoto, S., Sugiura, T., Waku, K., Takayama, H. and Kittaka, A., 2003. Efficient synthesis of 2-modified 1α,25-dihydroxy-19-norvitamin D3 with Julia Olefination: high potency in induction of differentiation on HL-60 cells. J Org Chem 68, 7407-7415.

Ponchon, G. and DeLuca, H.F., 1969. The role of the liver in the metabolism of vitamin D. J Clin Invest 48, 1273-1279.

Posner, G.H., Crawford, K.R., Yang, H.W., Kahraman, M., Jeon, H.B., Li, H., Lee, J.K., Suh, B.C., Hatcher, M.A., Labonte, T., Usera, A., Dolan, P.M., Kensler, T.W., Peleg, S., Jones, G., Zhang, A., Korczak, B., Saha, U. and Chuang, S.S., 2004. Potent, low-calcemic, selective inhibitors of CYP24 hydroxylase: 24-sulfone analogs of the hormone 1alpha,25-dihydroxyvitamin D3. J Steroid Biochem Mol Biol 89-90, 5-12.

Ramagopalan, S. V., Heger, A., Berlanga, A. J., Maugeri, N. J., Lincoln, M. R., Burrell, A., Handunnetthi, L., Handel, A.E., Disanto, G., Orton, S.M., Watson, C.T., Morahan, J.M., Giovannoni, G., Ponting, C.P., Ebers, G.C. and Knight, J.C., 2010. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res 20, 1352-1360.

Reeve L, Tanaka Y, DeLuca HF., 1983. Studies on the site of 1,25-dihydroxyvitamin D3 synthesis *in vivo*. J Biol Chem 258, 3615-3617.

Rhee, H.V., Coebergh, J.W. and Vries, E.D., 2009. Sunlight, vitamin D and the prevention of cancer: a systematic review of epidemiological studies. Eur J Cancer Prev 18, 458-475.

Ritter, C.S., Armbrecht, H.J., Slatopolsky, E. and Brown, A.J., 2006. 25-Hydroxyvitamin D(3) suppresses PTH synthesis and secretion by bovine parathyroid cells. Kidney Int 70, 654-659, Erratum in: Kidney Int 70, 1190, 2006.

Ritter, C.S. and Brown, A.J., 2011. Direct suppression of Pth gene expression by the vitamin D prohormones doxercalciferol and calcidiol requires the vitamin D receptor. J Mol Endocrinol 46, 63-66.

Rowland, P., Blaney, F.E., Smyth, M.G., Jones, J.J., Leydon, V.R., Oxbrow, A.K., Lewis, C.J., Tennant, M.G., Modi, S., Eggleston, D.S., Chenery, R.J. and Bridges, A.M., 2006. Crystal structure of human cytochrome P450 2D6. J Biol Chem 281, 7614-7622.

Sakaki, T., Sawada, N., Nonaka, Y., Ohyama, Y. and Inouye, K., 1999. Metabolic studies using recombinant escherichia coli cells producing rat mitochondrial CYP24: CYP24 can convert 1alpha,25-dihydroxyvitamin D3 to calcitroic acid. Eur J Biochem 262, 43-48.

Sansen, S., Yano, J.K., Reynald, R.L., Schoch, G.A., Griffin, K.J., Stout, C.D. and Johnson, E.F., 2007. Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2. J Biol Chem 282, 14348-14355.

Schlingmann, K.P., Kaufmann, M., Weber, S., Irwin, A., Goos, C., John, U., Misselwitz, J., Klaus, G., Kuwertz-Bröking, E., Fehrenbach, H., Wingen, A.M., Güran, T., Hoenderop, J.G., Bindels, R.J., Prosser, D.E., Jones, G. and Konrad, M., 2011. Mutations in CYP24A1 and Idiopathic Infantile Hypercalcemia. N Engl J Med 365, 410-421.

Schoch, G.A., Yano, J.K., Sansen, S., Dansette, P.M., Stout, C.D., and Johnson, E.F., 2008. Determinants of cytochrome P450 2C8 substrate binding: structures of complexes with montelukast, troglitazone, felodipine, and 9-cis-retinoic acid. J Biol Chem 283,17227-17237.

Schuster, I., 2011. Cytochromes P450 are essential players in the vitamin D signaling system. Biochim Biophys Acta 1814, 186-199.

Schwartz, G.G. and Hulka, B.S., 1990. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Res 10, 1307-1311.

Schwartz, G.G., Whitlatch, L.W., Chen, T.C., Lokeshwar, B. and Holick, M.F., 1998. Human prostate cells synthesize 1,25-dihydroxyvitamin D3 from 25-hydroxyvitamin D3. Cancer Epidemiol Biomark Prev 7, 391-395.

Semmler, E.J., Holick, M.F., Schnoes, H.K. and DeLuca, H.F., 1972. The synthesis of 1α,25-dihydroxycholecalciferol – a metabolically active form of vitamin D3. Tetrahedron Lett 40, 4147-4150.

Shinki, T., Shimada, H., Wakino, S., Anazawa, H., Hayashi, M., Saruta, T., DeLuca, H.F and Suda, T., 1997. Cloning and expression of rat 25-hydroxyvitamin D3-1alpha-hydroxylase cDNA. Proc Natl Acad Sci USA 94, 12920-12925.

Shinkyo, R., Sakaki, T., Kamakura, M., Ohta, M. and Inouye, K., 2004. Metabolism of vitamin D by human microsomal CYP2R1. Biochem Biophys Res Commun 324, 451-457.

Shultz TD, Fox J, Heath H 3rd, Kumar R., 1983. Do tissues other than the kidney produce 1,25-dihydroxyvitamin D3 *in vivo*? A reexamination. Proc Natl Acad Sci USA 80, 1746-1750.

Siegel, R., Naishadham, D. and Jemal, A. Cancer statistics, 2012. CA Cancer J Clin 62, 10-29.

Skowronski, R.J., Peehl, D.M. and Feldman, D., 1993. Vitamin D and prostate cancer: 1,25-dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines. Endocrinology 132, 1952-1960,

Skowronski, R.J., Peehl, D.M. and Feldman, D., 1995. Actions of vitamin D3, analogs on human prostate cancer cell lines: comparison with 1,25-dihydroxyvitamin D3. Endocrinology 136, 20-26.

St-Arnaud, R., Messerlian, S., Moir, J.M., Omdahl, J.L. and Glorieux, F.H., 1997. The 25-hydroxyvitamin D 1-alpha-hydroxylase gene maps to the pseudovitamin D-deficiency rickets (PDDR) disease locus. J Bone Miner Res 12, 1552-1559.

Strushkevich, N., Usanov, S.A., Plotnikov, A.N., Jones, G. and Park, H.W., 2008. Structural Analysis of CYP2R1 in Complex with Vitamin D3. J Mol Biol 380, 95-106.

Strushkevich, N., 2010. Structural basis of human CYP51 inhibition by antifungal azoles. J Mol Biol 397, 1067-1078.

Suda, T., DeLuca, H.F., Schnoes, H.K., Ponchon, G., Tanaka, Y. and Holick, M.F., 1970. 21,25-dihydroxycholecalciferol. A metabolite of vitamin D3 preferentially active on bone. Biochemistry 9, 2917-2922.

Swami, S., Krishnan, A.V. and Feldman, D., 2011. Vitamin D metabolism and action in the prostate: implications for health and disease. Mol Cell Endocrinol 347, 61-69.

Takeyama, K., Kitanaka, S., Sato, T., Kobori, M., Yanagisawa, J. and Kato, S., 1997. 25-Hydroxyvitamin D3 1alpha-hydroxylase and vitamin D synthesis. Science 277, 1827-1830.

Urushino, N., Yasuda, K., Ikushiro, S., Kamakura, M., Ohta, M. and Sakaki, T., 2009. Metabolism of 1alpha,25-dihydroxyvitamin D2 by human CYP24A1. Biochem Biophys Res Commun 384, 144-148.

Wang, L., Flanagan, J.N., Jamieson, D.P., Holick, M.F. and Chen, T.C., 2004. Regulation of 25-hydroxyvitamin D-1α-hydroxylase by epidermal growth factor in prostate cells. J Steroid Biochem Mol Biol 89-90C, 127-130.

Wang, L., Whitlatch, L.W., Flanagan, J.N., Holick, M.F. and Chen, T.C., 2003. Vitamin D autocrine system and prostate cancer. In: Reichrath, J., Friedrich, M. and Tilgen, W. (eds.) Recent Results in Cancer Research vol. 164: Vitamin D Analogs in Cancer Prevention and Therapy, Springer-Verlag Berlin Heidelberg, Germany, pp. 223-237.

Wang, L., Persons, K.S., Jamieson, D., Flanagan, J.N., Hsu, H.H., Holick, M.F., Luo, Z., Sakaki, T, Urushino, N., Faller, D.V. and Chen, T.C., 2008. Prostate 25-hydroxyvitamin D-1alpha-hydroxylase is up-regulated by suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor. Anticancer Res 28, 2009-2013.

Whitlatch, L.W., Young, M.V., Schwartz, G.G., Flanagan, J.N., Burnstein, K.L., Lokeshwar, B.L., Rich, E.S., Holick, M.F. and Chen, T.C., 2002. 25-Hydroxyvitamin D-1α-hydroxylase activity is diminished in human prostate cancer and is enhanced by gene transfer. J Steroid Biochem Mol Biol 81, 135-140.

Williams, P.A., Cosme,J., Sridhar, V., Johnson, E.F. and McRee, D.E., 2000. Mammalian microsomal cytochrome P450 monooxygenase: structural adaptations for membrane binding and functional diversity. Mol Cell 5, 121-131.

Williams, P.A., Cosme, J., Vinkovic, D.M., Ward, A., Angove, H.C., Day, P.J., Vonrhein, C., Tickle, I.J. and Jhoti, H., 2004. Crystal structures of human cytochrome P450 3A4 bound to metyrapone and progesterone. Science 305, 683-686.

Williams, P.A., Cosme, J., Ward, A., Angove, H.C., Vinković, D. M. and Jhoti, H., 2003. Crystal structure of human cytochrome P450 2C9 with bound warfarin. Nature 424, 464-468.

Wu, S., Ren, S., Nguyen, L., Adams, J.S. and Hewison, M., 2007. Splice variants of the CYP27b1 gene and the regulation of 1,25-dihydroxyvitamin D3 production. Endocrinology 148, 3410-3418.

Xu, W.S., Parmigiani, R.B. and Marks, P.A., 2007. Histon deacetylase inhibitors: molecular mechanisms of action. Oncogene 26, 5541-5552.

Yano, J.K., Hsu, M.H., Griffin, K.J., Stout, C.D. and Johnson, E.F., 2005. Structures of human microsomal cytochrome P450 2A6 complexed with coumarin and methoxsalen. Nat Struct Mol 12, 822-823.

Young, M.V., Schwartz, G.G., Wang, L., Jamieson, D.P., Whitlatch, L.W., Flanagan, J.N., Lokeshwar, B.L., Holick, M.F. and Chen, T.C., 2004. Prostate 25-hydroxyvitamin D-1α-hydroxylase is not influenced by parathyroid hormone and calcium: Implications for prostate cancer chemoprevention by vitamin D. Carcinogenesis 25, 967-971.

Key facts

- Esophageal cancer presents as either squamous cell carcinoma or adenocarcinoma, the latter of which develops via a pre-cursor condition called Barrett's esophagus.
- Incidence of Barrett's esophagus and esophageal adenocarcinoma is increasing in Western societies, particularly amongst white males.
- Contrastingly, esophageal squamous cell carcinoma is more common in developing countries and incidence rates are stable or declining.
- Obesity is a key risk factor for esophageal adenocarcinoma, whereas smoking and alcohol consumption are related to esophageal squamous cell carcinoma development.
- Current evidence suggests that vitamin D is not protective against esophageal carcinogenesis, and in fact may be associated with an increased risk of this cancer, particularly squamous cell carcinoma.

Summary points

- Vitamin D intake has been directly associated with esophageal adenocarcinoma risk, but not its pre-cursor conditions, and inversely associated with squamous cell carcinoma risk in European populations.
- No significant associations between vitamin D intake and esophageal cancer risk have been noted in the USA, where fortification of foods with vitamin D is prevalent.
- Being classified as vitamin D insufficient or deficient has been linked with a decreased risk of esophageal squamous cell carcinoma in Asian populations.
- No significant associations have been observed between vitamin D status and esophageal adenocarcinoma to date.
- Only small studies of vitamin D related genetic variants have been conducted in relation to esophageal cancer risk, none of which have identified potential polymorphisms at an altered risk of these lesions.
- The vitamin D receptor, through which vitamin D exerts its biological effects, has only been detected in Barrett's esophagus and adenocarcinoma tissue, and not squamous mucosa of the esophagus in limited laboratory investigations to date.
- The conflicting evidence of vitamin D and esophageal cancer published to date indicate a need for further research in this area in order to fully understand the mechanisms and true relationships that exist.

24. Vitamin D and esophageal cancer

H.G. Coleman, M.M. Cantwell and L.J. Murray
Cancer Epidemiology and Health Services Research Group, Centre for Public Health, Queen's University Belfast, Grosvenor Road, Belfast BT12 6BJ Northern Ireland, United Kingdom;
h.coleman@qub.ac.uk

Abstract

Esophageal cancer can present as two histological subtypes of esophageal cancer, adenocarcinoma or squamous cell carcinoma. The vast majority, if not all, esophageal adenocarcinomas arise from Barrett's esophagus. Similarly, squamous dysplasia carries an elevated risk of progression to esophageal squamous cell carcinoma. Both cancer types have extremely poor survival rates, and therefore there is an acute need to identify modifiable risk factors that may help to prevent their development. Despite the mechanistic and ecological support for a general anti-carcinogenic role of vitamin D, studies in relation to esophageal cancer have illustrated conflicting results. Studies of vitamin D intake have been inversely associated with squamous cell carcinoma risk and directly associated with adenocarcinoma risk in European studies, while no significant associations were observed in an American case-control study. Contradictory to this, evidence from high quality prospective cohorts have illustrated that low levels of circulating vitamin D are associated with a reduced risk of esophageal squamous cell carcinoma, particularly in Asian populations. Small studies of vitamin D related genetic variants have failed to detect an association with the risk of either histological subtype of esophageal cancer. Laboratory investigations do suggest, however, that vitamin D receptor expression is more apparent in Barrett's esophagus and esophageal adenocarcinoma tissue, and therefore are more likely to interact with vitamin D intake or status to impact on outcomes compared with squamous cell carcinomas. Whether these are positive or negative influences on development or survival remains unclear. Further work is clearly warranted in this area to fully understand the mechanisms involved and to clarify the conflicting evidence to date.

Keywords: esophageal adenocarcinoma, esophageal squamous cell carcinoma, Barrett's esophagus, dysplasia

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_24, © Wageningen Academic Publishers 2013

Abbreviations

CI	Confidence interval
HR	Hazard ratio
OR	Odds ratio
UV	Ultraviolet
VDR	Vitamin D receptor

24.1 Introduction

Esophageal cancer ranks 8th of the most common cancers worldwide in terms of incidence, but is accompanied by poor 5-year survival rates of approximately 10%, making it the 6th most common cause of cancer death globally (WCRF, 2007). The pathogenesis of esophageal cancer differs depending upon the location of the tumor. Cancers affecting the lower third of the esophagus tend to be adenocarcinomas that arise through a progressive spectrum of conditions resulting from repetitive gastro-esophageal reflux – namely reflux esophagitis, Barrett's esophagus and dysplasia. Conversely, tumors in the upper two thirds of the esophagus are usually squamous cell carcinomas. Both histological subtypes show different patterns of incidence, have distinctive risk factors and etiologies but are mutually associated with poor survival rates.

Squamous cell carcinomas of the esophagus historically comprised around 90% of all esophageal cancer cases, however rates of this histological sub-type are generally declining (Van den Brandt and Goldbohm, 2006). In contrast, esophageal adenocarcinoma incidence has approximately tripled over recent decades and it now accounts for around 50% of all new cases in Western countries (Van den Brandt and Goldbohm, 2006). Esophageal cancer predominantly occurs in males, and esophageal adenocarcinoma cases tend to occur more in white males whereas esophageal squamous cell carcinomas are more common in black males (Devesa *et al.*, 1998). These differing patterns of incidence provide an insight into risk factors that may influence these histological cancer subtypes.

Risk factors that are well documented for esophageal adenocarcinoma include male sex, gastro-esophageal reflux, increased body mass index, abdominal obesity and tobacco smoking, but not alcohol consumption (De Jonge *et al.*, 2007, Forman, 2004, Freedman *et al.*, 2011, Kim *et al.*, 1997, Lagergren, 2006), whereas both smoking and alcohol are related to esophageal squamous cell carcinoma etiology (Freedman *et al.*, 2007, Van den Brandt and Goldbohm, 2006). There are 'pockets' of epidemic areas of esophageal squamous cell carcinoma in developing countries, often related to specific habits. In northern Iran, an epidemic region is attributed to opium use and nass (a combination of tobacco, ash and lime) chewing, while intake of pickled vegetables (which are a source of mycotoxins and nitrosamines) contributes to high incidence rates in Linxian, China (Islami *et al.*, 2009, Kamangar *et al.*, 2009, Zheng *et al.*, 2010). Other nutritional components suggested to be protective against esophageal cancer include antioxidants, fruit and vegetables and dietary fiber (Anderson *et al.*, 2007, De Stefani *et al.*, 2008, Mulholland *et al.*, 2009)

Vitamin D has several generic anti-carcinogenic effects such as suppressing cell proliferation, promoting cell differentiation and regulating apoptosis (Dusso *et al.*, 2005). There is considerable evidence in the scientific literature citing a protective role for vitamin D against the development of many cancers (Giovannucci, 2009), and particularly colorectal cancer (Gorham *et al.*, 2007, Wei *et al.*, 2008). This chapter outlines the potential associations between esophageal cancer and vitamin D, evidence from observational studies conducted to date, and highlights gaps in our research knowledge.

24.2 Epidemiology of esophageal cancer and vitamin D: generating hypotheses

When esophageal cancer epidemiology is compared with that of vitamin D, it provides some interesting, but at times conflicting, hypotheses for potential associations. It is widely known that vitamin D sufficiency is increasingly inadequate in many populations, especially those residing away from the equator. For example, cases of rickets have re-emerged in the UK and Ireland, where vitamin D insufficiency has now been estimated to affect approximately 70% of the population (Hill *et al.*, 2004). Unlike in the USA, few dietary foodstuffs are routinely fortified with vitamin D in the UK and sunlight is too weak during winter months to stimulate cutaneous vitamin D synthesis. Such prevalent insufficiency rates are attributed to a variety of reasons, such as more widespread sun protection factor usage, an increase in computer usage and television watching resulting in more time being spent indoors, coupled with a reduction in physical activity undertaken outside. The UK has experienced some of the highest reported increases and absolute incidence rates of esophageal adenocarcinoma worldwide (Bosetti *et al.*, 2008, Gatenby *et al.*, 2011, Newnham *et al.*, 2003). Therefore, it could be postulated that vitamin D insufficiency is associated with esophageal adenocarcinoma development.

Exploration of racial disparities in esophageal cancer epidemiology provides further support for an anti-carcinogenic role of vitamin D. The manufacture of vitamin D from 7-dehydrocholesterol in the skin is much more limited in skin of darker pigmentation than Caucasian skin types (Mithal *et al.*, 2009). As noted above, the increase in esophageal adenocarcinoma incidence has been mainly pronounced in white males, rather than black males in whom esophageal squamous cell carcinoma remains the predominant histological subtype (Devesa *et al.*, 1998). Indeed, vitamin D insufficiency or deficiency prevalence is higher in African-American populations residing in the USA compared with Caucasian populations (Mithal *et al.*, 2009). This would suggest that vitamin D deficiency may also promote esophageal squamous cell carcinoma growth.

Alternative hypotheses would suggest that vitamin D is associated with a decreased risk of squamous cell carcinoma and/or an increased risk of adenocarcinoma development, or is of no etiological importance for this cancer:

- The relatively recent formulation and growing popularity of vitamin D supplement usage throughout the Western world parallel an increase in adenocarcinoma but not squamous cell carcinoma rates.

- Studies of second primary cancer risk in skin cancer patients, who are assumed to have superior long-term vitamin D status due to their UV exposure, have largely failed to identify an association with esophageal cancer risk.
- The male predominance of both histological subtypes of esophageal cancer cannot be clearly explained by patterns of vitamin D intake from foods or supplements, UV exposure or vitamin D insufficiency rates.

Overall the association, if any, between vitamin D and esophageal cancer is unlikely to be straightforward, and may involve interaction with other lifestyle or genetic risk factors. Evidence from observational studies investigating the role of vitamin D in esophageal tumorigenesis is summarized in the remainder of this chapter.

24.3 Vitamin D intake and esophageal cancer

A small number of case-control studies have investigated the association between dietary intake of vitamin D and esophageal cancer. A well designed American population-based study with approximately 300 cases of both esophageal adenocarcinoma and esophageal squamous cell carcinoma sought to investigate risk in relation to vitamin D intake (Mayne *et al.*, 2001). No significant associations were observed for both esophageal adenocarcinoma risk (OR 1.10; 95% CI: 0.86-1.40) and squamous cell carcinoma risk (OR 1.00; 95% CI: 0.74-1.36), when comparing the highest with the lowest category of vitamin D intake from foods (Mayne *et al.*, 2001).

More recent findings from an Irish population (Mulholland *et al.*, 2011) identified a significant two-fold increased risk of esophageal adenocarcinoma in those consuming the highest compared with the lowest intakes of vitamin D (OR 1.99; 95% CI: 1.03-3.86). The same study did not detect any associations between vitamin D intake and the risk of reflux esophagitis or Barrett's esophagus, which arise earlier in the carcinogenic pathway (Mulholland *et al.*, 2011). Conversely, the highest category of vitamin D intakes have been linked with a significant 42% reduced risk of esophageal squamous cell carcinoma in a series of Italian hospital-based case-control studies (Lipworth *et al.*, 2009). Similar inverse associations were also observed in a French case-control study (Launoy *et al.*, 1998).

Potential reasons for the conflicting findings from the studies outlined include the dietary assessment methods used and the different background populations studied. All studies used food frequency questionnaires, to assess diet, and the potential for dietary recall bias may have influenced results (Brown, 2006). Fortification programs operate in the USA, in which no significant associations were detected, and the richer vitamin D foodstuffs may not have been fully captured in the dietary assessment tools used (Calvo *et al.*, 2004). The palpable geographical and ethnic variation in the populations studied will also have had incurred differences in the vitamin D status, and related genetic variants, of the included participants that may impact upon the results observed (Davis, 2008).

In addition, it should be noted that no studies to date have specifically investigated vitamin D supplement intake and esophageal cancer risk. Data from observational studies is required in order to determine whether interventional studies are warranted in high risk population groups.

In summary, there is conflicting evidence from a limited number of studies investigating dietary vitamin D intake and esophageal cancer risk. European studies point towards a direct association between vitamin D intake and adenocarcinoma risk but an inverse association with squamous cell carcinoma risk. However, no significant associations were detected with either histological subtype in an American study.

24.4 Vitamin D status and esophageal cancer

Studies exploring vitamin D status, as measured by blood concentration levels of 25-hydroxyvitamin D, provide further intrigue to the association with esophageal cancer risk. Giovannucci and colleagues calculated predicted vitamin D status (on the basis of dietary and supplemental intake, skin pigmentation, adiposity, geographical location and physical activity) as part of a larger study of cancer incidence and mortality in men (Giovannucci *et al.*, 2006). In their analysis, a significant inverse association was observed between predicted vitamin D status and esophageal cancer risk, however this did not distinguish between histological subtypes of esophageal cancer.

This contrasts with studies comparing 545 esophageal squamous cell carcinoma patients (Chen *et al.*, 2007) and 230 squamous dysplasia cases (Abnet *et al.*, 2007) with appropriate controls residing in Linxian, China. In a cross-sectional analysis, the highest serum 25-hydroxyvitamin D concentrations were associated with significantly elevated risks of squamous dysplasia, which was evident in both men and women (Abnet *et al.*, 2007). These findings were reinforced with significantly increased risks of squamous cell carcinoma of the esophagus from prospective case-cohort analysis (HR 1.06; 95% CI: 1.01-1.13, per 15 nmol/l increment) (Chen *et al.*, 2007). These results were amongst the first to indicate that the widely published anti-carcinogenic effects of vitamin D may not be universally applied to all body sites.

Since the publication of the Chinese study results, a robust cohort consortium has sought to clarify the effects of circulating vitamin D concentrations in relation to rarer cancers. In fully adjusted analysis incorporating over 1000 cases, vitamin D status was not significantly associated with the risk of all upper gastrointestinal cancers (Abnet *et al.*, 2007). Compared to individuals with a vitamin D status in the reference range of 50-75 nmol/l, individuals with low circulating vitamin D concentrations did not have significantly altered risks of either squamous cell carcinoma or adenocarcinoma of the esophagus (Abnet *et al.*, 2007). Interesting observations were noted in subgroups analyses though, in which Asians and never smokers with the lowest vitamin D status had significantly reduced risks of upper gastrointestinal cancers overall. The authors concluded that increasing vitamin D status would not contribute to a lower risk of this cancer group (Abnet *et al.*, 2007).

In conclusion, results from studies to date suggest that if an association exists between vitamin D status and esophageal cancer risk, vitamin D insufficient or deficient states may actually serve to decrease risk. Evidence is most consistent for this observation in squamous cell carcinoma development in Asian populations.

24.5 Vitamin D receptor, related genetic variants and esophageal cancer

Vitamin D exerts its biological effects by binding to the VDR. Activation of the VDR induces changes in the expression of genes implicated in transcription, DNA synthesis, apoptosis and cell signaling of colon cancer cells (Palmer *et al.*, 2003). The impact of the VDR is less clear in esophageal tissue. VDR expression has been investigated in esophageal biopsies, in which no impact on apoptosis was observed (De Gottardi *et al.*, 2006). Furthermore, VDR expression did not differ between Barrett's esophagus, adenocarcinoma or normal mucosa samples investigated, although this investigation was restricted to only six biopsy samples per disease state (De Gottardi *et al.*, 2006). More recent data has found a change in VDR expression as the esophageal lining progresses from the native squamous epithelium to the columnar epithelium that indicates Barrett's esophagus (Trowbridge *et al.*, 2012a). In their study of 15 esophageal tumor specimens, Trowbridge and colleagues only detected VDR staining in the columnar metaplasia, and not normal squamous mucosa of the esophageal resections (Trowbridge *et al.*, 2012a,b). This would imply that vitamin D does not have an opportunity to bind locally in the esophagus, and therefore exert any potential anti-carcinogenic effects, unless the cell lining has undergone the metaplastic transition to Barrett's esophagus. This may explain why we do not observe a protective effect for vitamin D against squamous cell carcinoma of the esophagus, but does not clarify why vitamin D does not appear to hinder esophageal adenocarcinoma development (or, in fact, may increase risk). Although a tenuous suggestion, it may be that a substantive duration of Barrett's esophagus presence, combined with an adequate intake and/or circulating vitamin D status, is required in order to detect any interactions, if indeed one exists. Higher VDR expression was also associated with better response to neoadjuvant therapy, and therefore may be a suitable target for, improving survival from esophageal adenocarcinoma (Trowbridge *et al.*, 2012a,b).

VDR gene polymorphisms alter mRNA stability and transcriptional activity (Anderson *et al.*, 2003) and are related to tumor development (Holick *et al.*, 2007, Lowe *et al.*, 2005). A study in a Chinese population found no associations between VDR *TaqI* (C352T) genotypes and risk of esophageal squamous dysplasia (n=127 cases) or carcinoma (n=126 cases) (Li *et al.*, 2007). Similarly, no significant associations were identified in an Irish population-based case-control study of 224 esophageal adenocarcinoma cases, in which all of the common variation in the VDR gene polymorphisms were systematically investigated (Chang *et al.*, 2011). However, studies have generally been inadequately powered to study the association between vitamin D-related genetic polymorphisms and esophageal cancer outcomes (Chang *et al.*, 2011, Li *et al.*, 2007). In addition, vitamin D binding protein polymorphisms have recently been shown to be highly related to vitamin D status (Ahn *et al.*, 2010), and no studies to date have investigated their role in esophageal carcinogenesis.

In summary, given the potential relationship between low vitamin D status and an increased risk of esophageal cancer, there is an urgent need to fully elucidate the action of VDR and related genetic variants in this pathway in order to fully understand the mechanisms involved and to appropriately target dietary guidelines in future. Limited evidence to date suggests only Barrett's esophagus and esophageal adenocarcinoma express VDR, which is not observed in esophageal squamous mucosa, and therefore any potential effect of vitamin D, anti-carcinogenic or otherwise, may be limited to esophageal adenocarcinoma outcomes.

24.6 Conclusions

Better quality population-based studies are required to investigate the role of vitamin D intake, status, and related genetic variants in the esophageal cancer development, given the conflicting evidence to date. There is an accompanying need for further *in vitro* work to investigate the potential mechanisms involved.

References

Abnet, C.C., Chen, W., Dawsey, S.M., Wei, W.Q., Roth, M.J., Liu, B., Lu, N., Taylor, P.R. and Qiao, Y.L., 2007. Serum 25(OH)-vitamin D concentration and risk of esophageal squamous dysplasia. Cancer Epidemiol Biomarkers Prev 16, 1889-1893.

Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K.C., McCullough, M.L., Gallicchio, L., Jacobs, E.J., Ascherio, A., Helzlsouer, K., Jacobs, K.B., Li, Q., Weinstein, S.J., Purdue, M., Virtamo, J., Horst, R., Wheeler, W., Chanock, S., Hunter, D.J., Hayes, R.B., Kraft, P. and Albanes, D., 2010. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 19, 2739-2745.

Anderson, L.A., Watson, R.G., Murphy, S.J., Johnston, B.T., Comber, H., Mc Guigan, J., Reynolds, J.V. and Murray, L.J., 2007. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. World J Gastroenterol 13, 1585-1594.

Anderson, P.H., May, B.K. and Morris, H.A., 2003. Vitamin D metabolism: new concepts and clinical implications. Clin Biochem Rev 24, 13-26.

Bosetti, C., Levi, F., Ferlay, J., Garavello, W., Lucchini, F., Bertuccio, P., Negri, E. and La Vecchia, C., 2008. Trends in oesophageal cancer incidence and mortality in Europe. Int J Cancer 122, 1118-1129.

Brown, D., 2006 Do food frequency questionnaires have too many limitations? J Am Diet Assoc 106, 1541-1542.

Calvo, M.S., Whiting, S.J. and Barton, C.N., 2004. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr 80, 1710S-1716S.

Chang, C.K., Mulholland, H.G., Cantwell, M.M., Anderson, L.A., Johnston, B.T., McKnight, A.J., Thompson, P.D., Watson, R.G. and Murray, L.J., on behalf of the FINBAR study group, 2011. Vitamin D receptor gene variants and esophageal adenocarcinoma risk: a population-based case-control study. J Gastrointest Cancer, 43, 512-517.

Chen, W., Dawsey, S.M., Qiao, Y.L., Mark, S.D., Dong, Z.W., Taylor, P.R., Zhao, P. and Abnet, C.C., 2007. Prospective study of serum 25(OH)-vitamin D concentration and risk of oesophageal and gastric cancers. Br J Cancer 97, 123-128.

Davis,C.D., 2008. Vitamin D and cancer: current dilemmas and future research needs. Am J Clin Nutr 88, 565S-569S.

De Gottardi, A., Dumonceau, J.M., Bruttin, F., Vonlaufen, A., Morard, I., Spahr, L., Rubbia-Brandt, L., Frossard, J.L., Dinjens, W.N., Rabinovitch, P.S. and Hadengue, A., 2006. Expression of the bile acid receptor FXR in Barrett's esophagus and enhancement of apoptosis by guggulsterone *in vitro*. Mol Cancer 5, 48.

De Jonge, P.J.F., Wolters, L.M.M., Steyerberg, E.W., Van Dekken, H., Kusters, J.G., Kuipers, E.J. and Siersema, P.D., 2007. Environmental risk factors in the development of adenocarcinoma of the oesophagus or gastric cardia: a cross-sectional study in a Dutch cohort. Aliment Pharmacol Ther 26, 31-39.

De Stefani, E., Boffetta, P., Fagundes, R.B., Deneo-Pellegrini, H., Ronco, A.L., Acosta, G. and Mendilaharsu, M., 2008. Nutrient patterns and risk of squamous cell carcinoma of the esophagus: a factor analysis in Uruguay. Anticancer Res 28, 2499-506.

Devesa, S.S., Blot, W.J. and Fraumeni Jr, J.F., 1998. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 83, 2049-2053.

Dusso, A.S., Brown, A.J. and Slatopolsky, E., 2005. Vitamin D. Am J Physiol Renal Physiol 289, F8-28.

Forman, D., 2004. Review article: oesophago-gastric adenocarcinoma – an epidemiological perspective. Aliment Pharmacol Ther 20 Suppl 5, 55-60.

Freedman, N.D., Abnet, C.C., Leitzmann, M.F., Mouw, T., Subar, A.F., Hollenbeck, A.R. and Schatzkin, A., 2007. A prospective study of tobacco, alcohol, and the risk of esophageal and gastric cancer subtypes. Am J Epidemiol 165, 1424-1433.

Freedman, N.D., Murray, L.J., Kamangar, F., Abnet, C.C., Cook, M.B., Nyren, O., Ye, W., Wu, A.H., Bernstein, L., Brown, L.M., Ward, M.H., Pandeya, N., Green, A.C., Casson, A.G., Giffen, C., Risch, H.A., Gammon, M.D., Chow, W.H., Vaughan, T.L., Corley, D.A. and Whiteman, D.C., 2011. Alcohol intake and risk of oesophageal adenocarcinoma: a pooled analysis from the BEACON Consortium. Gut 60(8), 1029-1037.

Gatenby, P., Hainsworth, A., Caygill, C., Watson, A. and Winslet, M., 2011. Projections for oesophageal cancer incidence in England. 45, 184-185.

Giovannucci, E., Liu, Y., Rimm, E., Hollis, B., Fuchs, C., Stampfer, M. and Willett, W., 2006. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 98, 451-459.

Giovannucci, E., 2009. Vitamin D and Cancer Incidence in the Harvard Cohorts. Ann Epidemiol 19, 84-88.

Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M., Newmark, H.L., Giovannucci, E., Wei, M. and Holick, M.F., 2007. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. Am J Prev Med 32, 210-216.

Hill, T.R., O'Brien, M.M., Cashman, K.D., Flynn, A. and Kiely, M., 2004. Vitamin D intakes in 18-64-y-old Irish adults. Eur J Clin Nutr 58, 1509-1517.

Holick, C.N., Stanford, J.L., Kwon, E.M., Ostrander, E.A., Nejentsev, S. and Peters, U., 2007. Comprehensive association analysis of the vitamin D pathway genes, VDR, CYP27B1, and CYP24A1, in prostate cancer. Cancer Epidemiol Biomarkers Prev 16, 1990-1999.

Islami, F., Kamangar, F., Nasrollahzadeh, D., Moller, H., Boffetta, P. and Malekzadeh, R., 2009. Oesophageal cancer in Golestan Province, a high-incidence area in northern Iran – a review. Eur J Cancer 45, 3156-3165.

Kamangar, F., Chow, W., Abnet, C.C. and Dawsey, S.M., 2009. Environmental Causes of Esophageal Cancer. Gastroenterol Clin North Am 38, 27-57.

Kim, R., Weissfeld, J.L., Reynolds, J.C. and Kuller, L.H., 1997. Etiology of Barrett's metaplasia and esophageal adenocarcinoma. Cancer Epidemiol Biomarkers Prev 6, 369-377.

Lagergren, J., 2006. Etiology and risk factors for oesophageal adenocarcinoma: possibilities for chemoprophylaxis? Best Pract Res Clin Gastroenterol 20, 803-812.

Launoy, G., Milan, C., Day, N.E., Pienkowski, M.P., Gignoux, M. and Faivre, J., 1998. Diet and squamous-cell cancer of the oesophagus: a French multicentre case-control study. Int J Cancer 76, 7-12.

Li, H.Q., Diao, Y.T., Li, H., Zhou, Y.Z., Yang, Y.F., Fang, X.Q., Wang, Y., Wu, K., Zhao, D.L., Zhou, R.X. and Lei, F.H., 2007. The risk factors related to esophageal squamous cell cancer in Feicheng county, China. Chung Hua Yu Fang I Hsueh Tsa Chih 41, S56-61.

Lipworth, L., Rossi, M., McLaughlin, J.K., Negri, E., Talamini, R., Levi, F., Franceschi, S. and La Vecchia, C., 2009. Dietary vitamin D and cancers of the oral cavity and esophagus. 20, 1576-1581.

Lowe, L., Guy, M., Mansi, J., Peckitt, C., Bliss, J., Wilson, R. and Colston, K., 2005. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer 41, 1164-1169.

Mayne, S.T., Risch, H.A., Dubrow, R., Chow, W., Gammon, M.D., Vaughan, T.L., Farrow, D.C., Schoenberg, J.B., Stanford, J.L., Ahsan, H., West, A.B., Rotterdam, H., Blot, W.J. and Fraumeni, J.F., Jr, 2001. Nutrient intake and risk of subtypes of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 10, 1055-1062.

Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhardt, P., Dawson-Hughes, B., Eisman, J.A., El-Hajj Fuleihan, G., Josse, R.G., Lips, P., Morales-Torres, J. and IOF Committee of Scientific Advisors (CSA) Nutrition Working Group, 2009. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int 20, 1807-1820.

Mulholland, H.G., Cantwell, M.M., Anderson, L.A., Johnston, B.T., Watson, R.G., Murphy, S.J., Ferguson, H.R., McGuigan, J., Reynolds, J.V., Comber, H. and Murray, L.J., 2009. Glycemic index, carbohydrate and fiber intakes and risk of reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. Cancer Causes Control 20, 279-288.

Mulholland, H.G., Murray, L.J., Anderson, L.A., Cantwell, M.M. and FINBAR study group, 2011. Vitamin D, calcium and dairy intake, and risk of oesophageal adenocarcinoma and its precursor conditions. Br J Nutr 106, 732-741.

Newnham, A., Quinn, M.J., Babb, P., Kang, J.Y. and Majeed, A., 2003. Trends in the subsite and morphology of oesophageal and gastric cancer in England and Wales 1971-1998. Aliment Pharmacol Ther 17, 665-676.

Palmer, H.G., Sanchez-Carbayo, M., Ordonez-Moran, P., Larriba, M.J., Cordon-Cardo, C. and Munoz, A., 2003. Genetic signatures of differentiation induced by 1alpha,25-dihydroxyvitamin D3 in human colon cancer cells. Cancer Res 63, 7799-7806.

Trowbridge, R., Sharma, P., Hunter, W.J. and Agrawal, D.K., 2012a. Vitamin D receptor expression and neoadjuvant therapy in esophageal adenocarcinoma. Exp Mol Pathol 93, 147-153.

Trowbridge, R., Sharma, P., Hunter, W.J. and Agrawal, D.K., 2012b. Vitamin D receptor expression in the mucosal tissue at the gastroesophageal junction. Exp Mol Pathol 93(2), 246-249.

Van den Brandt, P.A. and Goldbohm, R.A., 2006. Nutrition in the prevention of gastrointestinal cancer. Best Pract Res Clin Gastroenterol 20, 589-603.

Wei, M.Y., Garland, C.F., Gorham, E.D., Mohr, S.B. and Giovannucci, E., 2008. Vitamin D and prevention of colorectal adenoma: a meta-analysis. Cancer Epidemiol Biomarkers Prev 17, 2958-2969.

World Cancer Research Fund/American Institute for Cancer Research, 2007. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. AICR, Washington DC, USA.

Zheng, S., Vuitton, L., Sheyhidin, I., Vuitton, D.A., Zhang, Y. and Lu, X., 2010. Northwestern China: a place to learn more on oesophageal cancer. Part one: behavioural and environmental risk factors. Eur J Gastroenterol Hepatol 22, 917-925.

Key facts

- Multiple sclerosis (MS) is an inflammatory disease of the central nervous system, in which auto-reactive T cells are involved in the pathology.
- A poor vitamin D status has been associated with a high risk of developing MS, and with an adverse outcome in MS.
- Vitamin D has extensive immune modulating properties *in vitro*, promoting immune homeostasis within the adaptive immune response.

Summary points

- Studies on immune modulating properties of vitamin D *in vivo* are highly heterogeneous in supplementation scheme of vitamin D and in the immunological outcomes measured.
- A trend towards an anti-inflammatory role of vitamin D appears to be present, but this impression is not substantiated by conclusive data.
- Studies on immune modulating potential of vitamin D *in vivo* should assess disease-specific relevant immunological outcomes in a controlled, well powered design.

25. Monitoring *in vivo* immune modulation by vitamin D in multiple sclerosis

A.-H. Muris[1,2,3], J. Damoiseaux[4] and J. Smolders[1,2,3,5]
[1]School for Mental Health and Neuroscience, Maastricht University Medical Center, Universiteitssingel 40, 6229 ER Maastricht, the Netherlands; [2]Department of Internal Medicine, Division of Clinical and Experimental Immunology, Maastricht University Medical Center, Universiteitssingel 50, 6229 ER Maastricht, the Netherlands; [3]Academic MS Center Limburg, Orbis Medical Center, Dr. H. van der Hoffplein 1, 6162 BG Sittard, the Netherlands; [4]Laboratory for Clinical Immunology, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, the Netherlands; [5]Neuroimmunology Research Group, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA Amsterdam, the Netherlands; smolders@gmail.com

Abstract

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system, characterized by an auto-reactive T cell response. High serum 25-hydroxyvitamin D levels have been associated with a decreased risk of developing MS and less severe disease. It is hypothesized that the underlying mechanism behind this association is the immune modulating effect of vitamin D on the adaptive immune response. *In vitro* studies and *in vivo* studies in the experimental auto-immune encephalomyelitis animal model have shown consistently that immune cells are functional targets of vitamin D. However, *in vivo* studies in humans are limited. In this chapter, studies on the relation between vitamin D and immunological parameters in healthy individuals and patients with different auto-immune diseases, including MS, are evaluated. Correlation studies and supplementation studies performed thus far are heterogeneous in many aspects and studied a diverse repertoire of immunological outcome measures. Overall, there seems to be a trend towards an anti-inflammatory role of vitamin D, but this impression is not substantiated by conclusive data. Well-powered and controlled studies assessing disease-specific relevant immunological outcomes are warranted before conclusions can be drawn. Clinical trials on vitamin D supplementation in MS and other diseases may provide excellent frameworks for such studies.

Keywords: auto-immune diseases, 25-hydroxyvitamin D, 1,25-dihydroxvitamin D, supplementation, correlation

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_25, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
APC	Antigen presenting cell
BD	Behcet's disease
BSA	Bovine serum albumine
CL	Cardiolipin
CNS	Central nervous system
CRP	C-reactive protein
CYP24A1	Cytochrome P24A1
CYP27B1	Cytochrome P27B1
DC	Dendritic cell
ICAM	Intracellular adhesion molecule
IFN	Interferon
IL	Interleukin
MBP	Myelin basic protein
MCP	Monocyte chemoattractant protein
MCTD	Mixed connective tissue disease
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
PBMC	Peripheral blood mononuclear cells
RRMS	Relapsing remitting multiple sclerosis
SLE	Systemic lupus erythematosus
TGF	Tumor growth factor
Th1	T helper cell type 1
Th17	T helper cell type 17
Th2	T helper cell type 2
TLR	Toll like receptor
TNFR	Tumour necrosis factor-α receptor
Treg	Regulatory T cell
VDR	Vitamin D receptor
Vitamin D2	Ergocalciferol
Vitamin D3	Cholecalciferol

25.1 Introduction

Since the beginning of the 20th century vitamin D has been particularly known for its function on bone and calcium metabolism. However, since the seventies, a poor exposure to vitamin D has also been increasingly noticed in and linked to the aetiology of MS (Goldberg *et al.*, 1986; Kurtzke, 1967). Since then, a new research field developed, addressing the implications of vitamin D in the pathophysiology of MS in epidemiological studies, intervention studies, *in vitro* studies

and animal studies (Smolders *et al.*, 2008a). Currently, almost 50 years later, first randomized controlled clinical trials are assessing the efficacy of vitamin D supplementation in MS. The view of vitamin D changed from that of a vitamin with a single role in calcium homeostasis, to that of an exciting steroid hormone involved in a multitude of cellular processes. One of these processes is the regulation of the adaptive immune response, with many *in vitro* and animal studies showing important regulatory effects of vitamin D (Hayes *et al.*, 1997; Smolders and Damoiseaux, 2011). One of the major challenges that lies ahead is to understand if and how systemic vitamin D status interferes with the human adaptive immune response *in vivo*. Clarification of the interaction between the availability of vitamin D at tissue level (target and secondary lymphoid organs) and the extent to which an immune response is initiated or thriven, may reveal underlying mechanism for associations between vitamin D status and MS.

The aim of this chapter is not to give an extensive overview of the current knowledge about either the immune dysregulation in MS, or the association between vitamin D and MS, or the role of vitamin D in the immune response at a cellular level. Since these have been reviewed extensively by us and others, we will refer to these reviews were appropriate. In the present chapter, we will focus on studies exploring the *in vivo* relevance of the interaction between vitamin D and the immune response. To achieve this, we will discuss studies on correlations between vitamin D status and relevant immune outcome parameters, and studies on immunological outcome measures in vitamin D supplementation trials, both in healthy controls as well as in patients with auto-immune disease including MS. We will integrate these findings and discuss considerations for the design of trials on the immune modulating effects of vitamin D in MS.

25.2 Multiple sclerosis

Multiple sclerosis is an inflammatory, demyelinating disease of the CNS. The prevalence is 30-100 per 100,000 individuals (Milo and Kahana, 2010; Rosati, 2001). It is twice as common in females than in males, with an incidence between 20-50 years, and a peak incidence at 30 years of age (Compston and Coles, 2002; 2008). MS patients have a heterogeneous disease presentation. In RRMS, periods without neurological exacerbations are altered by periods of increased neurological symptoms. These relapses can be characterised by symptoms of loss of motor and/ or sensory function, lack of coordination, but also mental and physical fatigue and/ or cognitive decline. After several years, 65% of the RRMS patients becomes secondary progressive MS and they experience constant neurological deterioration. A small fraction of the patients experience a progressive accumulation of disability from the beginning of the disease onwards, called primary progressive MS (Compston and Coles, 2002; 2008).

The exact aetiology of MS is still unknown, but one of the main hypotheses is that multiple sclerosis is a T-cell mediated auto-immune disease. This model for MS has been reviewed by several authors (Bar-Or, 2008; Milo and Kahana, 2010). Shortly, it is thought that auto-reactive lymphocytes are primed with an unknown auto-antigen in the secondary lymphoid organs by APCs. Clonally expanded and activated effector T cells migrate through the blood brain barrier

into the perivascular space. Here, the T cells are reactivated by APCs, like perivascular macrophages or DCs, and migrate into the brain parenchyma. In the parenchyma, the T cells contribute to an inflammatory response in which loss of oligodendrocytes and myelin occurs, often followed by loss of neurons. It is a matter of debate whether MS is truly a classical auto-immune disease, with an auto-reactive response directed against a single auto-antigen (Bar-Or, 2008). Several lines of evidence suggest a general lack of control of the T cell response in MS. Firstly, many genetic polymorphisms associated with MS are involved in the maintenance of T cell homeostasis (Sawcer *et al.*, 2011). Secondly, several differences in general characteristics of the circulating T cell compartment have been found between MS and control patients. During active disease, increased proportions of pro-inflammatory Th1 and Th17 cells, as well as increased transcription of IL-17 mRNA have been described (Durelli *et al.*, 2009; Edwards *et al.*, 2010; Frisullo *et al.*, 2008; Matusevicius *et al.*, 1999). Most notably, the capacity of Treg to suppress polyclonal or antigen-specific proliferation of effector T cells was reduced in MS (Haas *et al.*, 2005; Venken *et al.*, 2006; Viglietta *et al.*, 2004). In addition, a reduced transcription of Treg-related genes by PBMC (Dalla Libera *et al.*, 2011; Edstrom *et al.*, 2011), and a reduction of Treg-subsets with specific surface molecules as CD39 (Borsellino *et al.*, 2007) have been described. Interestingly, these $CD39^+$ Tregs are able to suppress IL-17 production by activated T cells (Fletcher *et al.*, 2009). Accordingly, our group found a negative correlation between the proportion of $CD39^+$ Treg and the proportion of Th17 cells in the circulation of RRMS patients (Peelen *et al.*, 2011a). Thirdly, the CNS tissue of MS patients is infiltrated with $CD4^+$ but predominantly $CD8^+$ T cells (Frischer *et al.*, 2009). The clonality of these cells is diverse, and does not differ between lesions and normal appearing white matter (Junker *et al.*, 2007). Beside T cells, B cells are also found in increased number in the CNS of MS patients (Frischer *et al.*, 2009). Interestingly, oligoclonal intrathecal IgG-production against predominantly several myelin proteins and heat shock proteins is a hallmark of the disease process (Quintana *et al.*, 2012). Lastly, currently registered MS disease modulating therapies interfere with either phenotypic and functional characteristics of circulating T cells (Cohen *et al.*, 2010; Noronha *et al.*, 1993), migration of lymphocytes into the CNS (Polman *et al.*, 2006), or deplete B and T cells from the circulation (Coles *et al.*, 2008; Hauser *et al.*, 2008).

25.3 Multiple sclerosis and vitamin D

Several epidemiologic and genetic studies show that a poor exposure to vitamin D is a putative risk factor for developing MS, as has been reviewed elsewhere (Ascherio *et al.*, 2010; Smolders *et al.*, 2008a). In brief, genetic polymorphisms within key enzymes involved in vitamin D metabolism, as well as within the VDR, have been associated with the risk on developing MS (Sawcer *et al.*, 2011; Smolders *et al.*, 2009a), as well as a poor vitamin D status prior to disease onset (Munger *et al.*, 2006). In established MS, a poor vitamin D status is associated with a high risk of relapses (Mowry *et al.*, 2010; Simpson *et al.*, 2010; Smolders *et al.*, 2008b) and a high MS-related disability score (Smolders *et al.*, 2008b; van der Mei *et al.*, 2007). Supplementation of high doses of vitamin D3 in MS patients did not provoke hypercalcaemia and was well tolerated in several pilot studies (Burton *et al.*, 2010; Kimball *et al.*, 2007; Smolders *et al.*, 2010b). A pilot study in MS on treatment with the biologically active metabolite of vitamin D, $1,25(OH)_2D3$, reported the development

of a hypercalcaemia in 2/15 subjects (Wingerchuk *et al.*, 2005). Exploratory analyses on clinical endpoints suggested a reduction of MRI activity in an uncontrolled study (Kimball *et al.*, 2007), a reduction of relapse activity in an uncontrolled (Smolders *et al.*, 2010b) and controlled study (Burton *et al.*, 2010), and a reduction of disability progression in a controlled study design (Burton *et al.*, 2010). A study supplementing vitamin D2, did not show clear difference on primary MRI endpoints between the high- and low-dose treatment arms (Stein *et al.*, 2011). Subsequently, first clinical trials are currently being performed to assess the efficacy of high dose vitamin D3 supplementation in MS (Dorr *et al.*, 2012; Smolders *et al.*, 2011). Although vitamin D appears to be implicated in the disease process of MS, the underlying mechanism is not known.

25.4 Vitamin D metabolism

Vitamin D is the precursor of an essential hormone (Lips, 2006). Its natural sources are diet, both from vegetable origin (ergocalciferol or vitamin D2) as well as from animal origin, especially fatty fish (cholecalciferol or vitamin D3), and most importantly synthesis in the skin under the influence of ultraviolet B radiation in sunlight (90-100%). The epidermal layer of the skin contains 7-dehydrocholesterol which is transformed into pre-vitamin D3 under influence of ultraviolet B radiation and body temperature. Subsequently, most of the vitamin D metabolites in the circulation are of vitamin D3 origin and only a negligible part of vitamin D2. In the circulation and tissues, vitamin D is mostly present as 25(OH)D, which is formed in the liver. The circulating levels of this metabolite are most useful to describe an individual's vitamin D status, although 25(OH)D is not biologically active. A second hydroxylation step is needed to obtain the biologically active metabolite of vitamin D, $1,25(OH)_2D$. This step is catalyzed by the enzyme cytochrome P27B1 (CYP27B1 or 25(OH)D-1α hydroxylase) (Dusso *et al.*, 2005). $1,25(OH)_2D$ in the circulation is almost exclusively produced by the kidney (Jongen *et al.*, 1984), and circulating levels are regulated by the endocrine control of calcium homeostasis (Lips, 2006). However, many other tissues can 'activate' 25(OH)D (Zehnder *et al.*, 2001). Once activated, $1,25(OH)_2D$ behaves as a steroid hormone. It binds to the intracellular VDR which regulates the transcription of more than 500 genes containing the vitamin D response elements. Catabolism of $1,25(OH)_2D$ is regulated by the enzyme CYP24A1 ($1,25(OH)_2D$ 24-hydroxylase) (Holick, 2007). Several cut-off points for an optimal vitamin D status have been proposed, mostly based on its role in the endocrine control of calcium homeostasis (Holick, 2007; Holick *et al.*, 2011; Institute of Medicine, 2010; Ross, 2011; Vieth, 1999). Since the relevance of these cut-off values for other biological functions of vitamin D are uncertain, they are not very useful in research.

25.5 Vitamin D as an immune modulator

All immune cells involved in the adaptive immune response have the potential to metabolize and to respond to vitamin D, as has been reviewed extensively (Baeke *et al.*, 2010; Peelen *et al.*, 2011b; Smolders and Damoiseaux, 2011). Shortly, resting and activated myeloid cells, including monocytes, macrophages and DCs, and activated T- and B-lymphocytes express the

VDR (Chen *et al.*, 2007; Penna *et al.*, 2007; Provvedini *et al.*, 1983; Veldman *et al.*, 2000). In lymphocytes, exposure to $1,25(OH)_2D$ further enhances VDR expression (Chen *et al.*, 2007; Veldman *et al.*, 2000). Myeloid APCs, and most notably the specialized DCs, lose their antigen presenting potential upon exposure to $1,25(OH)_2D$, and skew towards an immature innate profile (Penna and Adorini, 2000). Subsequently, they have less capability to start a T cell response (Van Halteren *et al.*, 2002). Accordingly, $1,25(OH)_2D$ inhibits T cell proliferation and the production of pro-inflammatory cytokines as IFN-γ and IL-17 (Correale *et al.*, 2009). The synthesis of anti-inflammatory cytokines, such as IL-4 and IL-10, is promoted. Besides being targets of vitamin D, activated myeloid cells (either activated by adaptive or innate stimuli) and lymphocytes (either activated polyclonally or via antigen specific stimulation) express the activating enzyme CYP27B1, and subsequently synthesize $1,25(OH)_2D$ locally (Correale *et al.*, 2009; Fritsche *et al.*, 2003; Gottfried *et al.*, 2006; Heine *et al.*, 2008; Penna *et al.*, 2007; Sigmundsdottir *et al.*, 2007). All these cells also up-regulate the catabolic enzyme CYP24A1 upon exposure to $1,25(OH)_2D$ (Correale *et al.*, 2009; Gottfried *et al.*, 2006; Penna *et al.*, 2007). Since these observations are done *in vitro*, it is uncertain whether these dynamics also apply to immune activation within tissues where the immune response becomes manifest, i.e. the secondary lymphoid tissues as well as the peripheral tissues affected by a disease. Nevertheless, studies in the experimental MS model auto-immune encephalomyelitis showed an up-regulation of VDR and CYP27B1 and a down-regulation of CYP24A1, together with an enhanced local $1,25(OH)_2D$ production within the inflamed CNS (Spach and Hayes, 2005). Accordingly, supplementation of vitamin D or treatment with $1,25(OH)_2D$ prevented development of experimental auto-immune encephalomyelitis and ameliorated the disease course (Cantorna *et al.*, 1996; Lemire and Archer, 1991). Both in the circulation, lymph nodes, and CNS, proliferation and cytokine assays suggested a shift of the immune response towards a less inflammatory profile (Smolders *et al.*, 2008a).

25.6 Vitamin D status, vitamin D supplementation and the effect on immune regulation *in vivo*

Since an auto-reactive T cell response has been implicated in the disease process of MS, an immune modulating, dampening effect of vitamin D may be very relevant (Hayes *et al.*, 1997). Although effects of vitamin D metabolites on immune cells *in vitro* have been frequently investigated, *in vivo* data are limited. The negative correlation between vitamin D status and the risk on developing MS was observed within the physiologically occurring ranges of vitamin D, which is 0-150 nmol/l (Munger *et al.*, 2006). Within the same range of 25(OH)D, a negative correlation between vitamin D status and MS relapse rate was found (Mowry *et al.*, 2010; Simpson *et al.*, 2010; Smolders *et al.*, 2008b). If the driving force of these correlations is the promotion of T cell homeostasis within the higher ranges of serum 25(OH)D, relevant measures of immune-regulation would be expected to correlate positively with vitamin D status *in vivo*.

We conducted a literature search to identify papers which investigated either the correlation of serum 25(OH)D levels with measures of immune homeostasis, or the effect of vitamin D supplementation on immune parameters determined in peripheral blood (correlation studies

are summarized in Table 25.1, supplementation studies in Table 25.2). We focused on studies in healthy controls, patients with MS, and patients with other auto-immune diseases. Since the systemic metabolism of vitamin D is disturbed in kidney disease patients, we excluded studies done in this patient population. Furthermore, studies in cancer patients, HIV patients, allergic patients and patients with an infectious disease were excluded, because the interaction of vitamin D with the immune system may be different from auto-immune diseases due to distinct immunopathology. Moreover only studies in which 25(OH)D levels were measured and reported were included. Epidemiological studies addressed association between serum 25(OH)D levels, and the efficacy of vitamin D2/D3 supplementation (*vide supra*). Additionally, while the effect of vitamin D on the immune system is most likely mainly executed by local activation of vitamin D, this is rather dependent on circulating 25(OH)D than on $1,25(OH)_2D$ levels. Therefore only supplementation studies in which vitamin D2 or D3 was supplemented and in which the effects of vitamin D supplementation could be distinguished from other possible co-interventions, were included. Since several approaches have been used to evaluate the effect of vitamin D on the immune function *in vivo*, we will discuss the different immunological outcome measures that have been used in these studies.

25.6.1 Inflammatory markers in the circulation

First of all, several groups addressed correlations of serum 25(OH)D with serum concentrations of general markers of inflammation, chemokines, and cytokines. Serum concentrations of CRP did not correlate with serum 25(OH)D in most studies with controls (Peterson and Heffernan, 2008; Shea *et al.*, 2008; Vilarrasa *et al.*, 2010). Supplementation of vitamin D did also not influence CRP levels in healthy, older or obese persons (Jorde *et al.*, 2010; Pittas *et al.*, 2007; Prietl *et al.*, 2010) or in patients with MS (Kimball *et al.*, 2011). However, a negative correlation between these parameters has been described in patients with SLE (Thudi *et al.*, 2008), ankylosing spondylitis or psoriatric arthritis (Teichmann *et al.*, 2010) and BD (Do *et al.*, 2008; Hamzaoui *et al.*, 2010). Regarding complement, a study among healthy individuals reported higher serum concentrations of both 25(OH)D and C3 after a long-distance holiday (Falkenbach and Sedlmeyer, 1997), and higher serum 25(OH)D levels correlated with higher serum C4 in subjects with SLE (Szodoray *et al.*, 2011).

25.6.2 Cytokines and chemokines in the circulation

In healthy controls, multiple studies reported no association of higher serum 25(OH)D levels with serum concentrations of pro-inflammatory cytokines as IL-6 (Peterson and Heffernan, 2008; Shea *et al.*, 2008), IL-12 (Hajas *et al.*, 2011), IL-17 (Hajas *et al.*, 2011), IL-18 (Vilarrasa *et al.*, 2010), IFN-γ (Hajas *et al.*, 2011), TNF-α (Shea *et al.*, 2008) and the anti-inflammatory cytokine IL-10 (Peterson and Heffernan, 2008). Accordingly, no correlations were reported with the immune-related molecules soluble (s)CD40L (Shea *et al.*, 2008), sP-selectin (Shea *et al.*, 2008), osteoprotegerin (Shea *et al.*, 2008), sTNFR1 (Vilarrasa *et al.*, 2010), sTNFR2 (Shea *et al.*, 2008; Vilarrasa *et al.*, 2010), sICAM-1 (Shea *et al.*, 2008) and MCP-1 (Shea *et al.*, 2008). Likewise, supplementation studies with vitamin D in healthy controls and MS patients showed

Table 25.1. Cross sectional and cohort studies on 25(OH)D levels *in vivo* and immunological outcome measures in peripheral blood in health and auto-immune disease; the relation of 25(OH)D to the outcome measures are given as ↑: increased; ↓: decreased; =: not affected.

Study		Outcome measures	
Study design	**Study population (n)[1]**	**25(OH)D (nM) (mean or median)**	**Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)**
Healthy subjects			
CH (Falkenbach and Sedlmeyer, 1997)	Healthy, long distance holiday (33)	85.4 (±44.7) to 110 (±37.6)	Circulating total blood count =, $CD4^+$ cells =, $CD8^+$ cells =, circulating $CD4^+/CD8^+$ T cell ratio ↑, serum IgG ↓, IgA ↑, IgM =, C3 ↑, sIL-2R ↑, soluble CD14 ↑
CS (Feser *et al.*, 2009)	Predicted RA in future (76 cases)	67.1±25.1	Serum no corr. RF-IgM, RF-IgG, RF-IgA, anti-CCP
	Controls at risk for RA or DMI (154)	63.1±22.5	
CS (Jorde *et al.*, 2010)	28.0≤BMI≤47.0 kg/m^2 (37)	56 (15-136)	Serum MCP-1 (♂): R^2=-0.28 (P<0.01), MCP-1 (♀): R^2=+0.2 (P<0.01), IL-5 (♀): R^2=-0.16 (P<0.05), IL-10 (♀): R^2=-0.14 (P<0.05)
CH (Khoo *et al.*, 2011)	Healthy, ♂ (15)		Supernatants IL-1β ↓, IL-6 ↓, IL-10 ↓, TNF-a ↓, IFN-γ ↓ (PBMC+LPS)
	Winter to spring	43	
	Summer	89	Supernatants IL-1β ↓/=, IL-6 =, IL-10 =, TNF-a ↓ (PBMC+Pam3Cys)
	Autumn	±70	
			Circulating monocytes TLR-2 ↓, TLR-4 ↓
CS (Peterson and Heffernan, 2008)	Healthy, ♀		Serum CRP =, IL-6 =, IL-10 =, TNF-a ↓
	high 25(OH)D (20)	129.6±11.0	Serum TNF-a: R^2= -0.06 (P<0.05), no corr. CRP, IL-6, IL-10
	low 25(OH)D (49)	74.4±4.0	
CS (Shea *et al.*, 2008)	Birth cohort, mean intake vit.D 426 IU/d (1,381)	49.4±18.6	Serum no corr. CRP, IL-6, CD40L, P-selectin, osteoprotegerin, TNF-a, sTNFR2, sICAM-1, MCP-1
			Serum IL-6 R=neg. (P=0.01) (subgroup non-cardiovascular patients)
CS (Vilarrasa *et al.*, 2010)	Healthy (261)	52.7±21.3	Serum no corr. CRP, IL-18, sTNFR1, sTNFR2
	Obese (44)	37.8±16.5	
CS (Zittermann *et al.*, 2004)	Newborns		Cord blood IL-10 ↑, IgE ↓/=
	Summer (49)	52.2 (29.5-65.3)	Cord blood IL-10: R=+0.22 (P<0.05), no corr. IgE
	Winter (47)	26.2 (18.3-42.5)	

Table 25.1. Continued.

Study		Outcome measures	
Study design	**Study population (n)[1]**	**25(OH)D (nM) (mean or median)**	**Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)**
Auto-immune disease			
CS (Ben-Zvi *et al.*, 2010)	SLE (19)	range <49.9, >74.9	Circulating mDC/pDC numbers =, circulating DC HLA-DR =, CD40 =, CD86 =
	African Americans	35.4 (22.7-47.4)	
	Hispanics	51.2 (32.4-74.9)	
	Asians	54.9 (28.7-73.6)	
	Caucasians	72.4 (36.2-84.9)	
CS (Bonakdar *et al.*, 2011)	SLE, ♀ (40)	24.2±2.1 (7.9-78.0)	Serum anti-dsDNA ↓
		(range 25-39.9, 12.5-24.9, <12.5)	
CS (Do *et al.*, 2008)	BD active (23)	27.2±10.8	Serum CRP: R=-0.37 (P<0.05)
	BD inactive (18)	31.1±9.1	Circulating $CD14^+$ monocytes TLR2: R=-0.37 (P<0.05); TLR4 R=-0.43 (P<0.05)
	Psoriasis control (19)	37.2±15.2	
	Healthy (15)	35.0±8.9	No corr. circulating $CD16^+$ $CD14^+$ cells
CS (Hajas *et al.*, 2011)	MCTD, ♀ (125)	65.3±33.7	Serum anti-U1-RNP: R=-0.246 (P=0.02), anti-CL IgA: 0.396 (P=0.02)
	Healthy (48)	87.2±24.1	Serum no corr. CRP, anti-CL IgG/ IgM, AECA, IL-12, IL-17, IFN-γ
			Serum IL-23: R= -0.473 (P=0.002), IL-6: R=-0.618 (P<0.001), IL-10: R=0.338 (P<0.004)
CS (Hamzaoui *et al.*, 2010)	BD active (102)	22.5±14,1	Serum CRP: R=-0.3634 (P<0.001) (whole population)
	BD inactive (58)	27.7±12.9	
	RA control (22)	14.1±5.5	Circulating IFN-γ^+CD4^+ T cells: R=-0.55 (P=0.012), IFN-γ/IL-4 ratio: R=-0.599 (P=0.005), IL-17^+ cells: R=-0.462 (P=0.040), Treg: R=0.640 (P=0.002), IL-10: R=0.808 (P=0.0001) (subpopulation of active BD n=20)
	MS control (30)	17.0±9.1	
	Healthy (50)	35.0±13.0	

Table 25.1. Continued.

Study		Outcome measures	
Study design	**Study population (n)[1]**	**25(OH)D (nM) (mean or median)**	**Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)**
CS (Kivity *et al.*, 2011)	AITD and non-AITD		Serum anti thyroid peroxidise ab ↓, anti thyroid globulin ab ↓, anti thyroid stimulating hormone receptor ab ↓
	High 25(OH)D (34)	42.4±15.0 (25.0-104.8)	
	Low 25(OH)D (58)	18.5±2.5 (17.5-25.0)	
	Healthy (98)		
CS (Ritterhouse *et al.*, 2011)	SLE, ♀ (32)	43.2 (29.7-52.9) (range <49.9, >49.9)	Circulating B cell activation (pERK 1/2) ↓, pERK1/2: R=-0.40 (P0.03) (SLE)
	Healthy (32)		Serum anti-SS-A ↓/=, ANA ↓, no corr anti-dsDNA, anti-SS-B, anti-Sm, anti-nRNP, anti-ribo P, aPL
	ANA pos controls	43.4 (36.2-64.4)	
	ANA neg. controls	±69.9	Serum IFN-a activity: R^2=+ (*P*=0.03), anti-Ro, anti-dsDNA & anti-nRNP specificity R^2=+0.138 (*P*=0.037)
CS (Szodoray *et al.*, 2011)	SLE (177)	67.1±33.1 (range >74.9, 37.4-74.9, <37.4)	Serum IgG ↓, C4 ↑, anti-dsDNA ↓, ANA ↑, Serum anti-Sm: R=0.288 (*P*<0.001)
CS (Teichmann *et al.*, 2010)	ASP (76)	24.9±32.1	Serum CRP: R=-0.350 (*P*<0.001), anti-htTG IgA ↓
	PsA (116)	48.4±46.4	
CS (Thudi *et al.*, 2008)	SLE, ♀ (37)	76.6± 6.67 (range <47.7, >47.7)	Serum CRP =, ANA =, ↑ anti-dsDNA Serum CRP: R=-0.31 (*P*=0.09)
CS (Knippenberg *et al.*, 2011)	RRMS remission (23)	107.0 (77.0-128.0)	No corr. circulating B cell s, memory B cells, naive B cells, Breg
	RRMS relapse (22)	66.5 (47.3-100.1)	
	Healthy (30)	87.5 (67.5-95.0)	
CS (Peelen *et al.*, 2011a)	RRMS remission (32)	79.5 (63-107)	No corr. circulating $IL17^+CD4^+$T cells, trend towards lower vit D in patients with expansion Th17 cells
	RRMS relapse (22)	64 (49-89)	
	Healthy (30)	87.5 (68-95)	Circulating $CD39^+$ Treg (remission): R=−0.399 (*P*=0.024), not in MS relapse or healthy

Table 25.1. Continued.

Study		Outcome measures	
Study design	**Study population (n)[1]**	**25(OH)D (nM) (mean or median)**	**Immunological outcome measures (between groups (high versus low 25(OH)D) and correlations)**
CS (Royal *et al.*, 2009)	RRMS (26)	80.5±7.4	Circulating Treg: R=-0.44 (*P*=0.02), no corr. total naïve/memory CXCR3+ T cells or ratio
	Treated		
	Untreated	73.5±6.9	Untreated patients: circulating CXCR3+ naïve T cell R=0.49 (*P*=0.027), CXCR3+ naïve/memory T cell ratio R=0.61 (*P*=0.004)
CS (Smolders *et al.*, 2009, 2010a)	RRMS (29)	54 (19-133)	No corr. circulating Th1 (R=0.34 (*P*=0.060)) %Th2, %IL-10+CD 4^+ T cells, circulating Tregs Circulating Th1/Th2 ratio: R=-0.44 (*P*=0.023) Circulating Treg suppressive capacity: R=-0.590 (*P*=0.002)

[1] Study population in which relevant immunological outcome measure with respect to 25(OH)D levels were measured.

Abbreviations: 25(OH)D = 25-dihydroxy vitamin D; ab = antibody; AECA = anti-endothelial cell antibody; AITD = auto-immune thyroid diseases; ANA = antinuclear antibody; ASP = ankylising spondylitis; BD = Behcet's disease; BMI = body mass index; Breg = regulatory B cell; C = complement component; CCP = cyclic citrullinated peptide; CH = cohort; CL = cardiolipin; CRP = C-reactive protein; CS = cross-sectional; DC = dendritic cell; DM = diabetes mellitus; dsDNA = double stranded DNA; htTG = human anti-tissue-transglutaminase; ICAM = intercellular adhesion molecule; IL = interleukine; MCP = monocyte chemoattractant protein; MCTD = mixed connective tissue disease; MS = multiple sclerosis; nRNP = nuclear ribonucleoprotein; RA = rheumatoid arthritis; RRMS = relapsing remitting multiple sclerosis; pERK = phospho-Erk; PL = phospholipid; PsA = psoriatic arthritis; ribo P = ribosomal P; RF = rheumatoid factor; RNP = ribonucleoprotein; SLE = systemic lupus erythomatosus; Sm = Smith antigen; Th = T-helper; TLR = Toll-like receptor; TNFR = tumour necrosis factor-a receptor; Treg = regulatory T cell; ♂ = male; ♀ = female.

Table 25.2. Vitamin D supplementation studies and immunological outcome measures in peripheral blood in health and auto-immune disease; the effect on the outcome measures is given as ↑: increased; ↓: decreased; =: not affected.

Study			Outcome measures	
Study design (duration)	**Study population (n) + vitamin[1]**	**Dose (with weekly equivalents)**	**Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation)**	**Immunological outcome measures[2]**
Healthy subjects				
OL (5 wks) (Adams *et al.*, 2009)	Healthy low BMD & 25(OH)D (7) D2	2 times 50,000 IU/w (100,000)	61.7±10.2 to 100.6±24.2	Circulating monocyte hCAP mRNA ↑
OL (15 wks) (Allen *et al.*, 2012)	Healthy (4) D3	5,000 IU/d for 10 wks followed by 5,000/10,000 for 5 wks	38 (27-53) to 179.5	Supernatants IL-10 ↑ (PBMC+PPD/TT/DT/PHA) ↓ IL-17$^+$ CD4$^+$ T cells, = IL-10$^+$ CD4$^+$ T cells, = IL-10$^+$ CD8$^+$ T cells (PBMC+PHA) Supernatants IL-10 ↑ (total PBMC & CD4^{depleted} PBMC), IL-10= (CD4$^+$PBMC); IL-17 ↓ (total PBMC & CD4$^+$ PBMC), IL-17= (CD4^{depleted}PBMC) (sorted PBMC+PHA)
RCT, PC (22 wks) (Barnes *et al.*, 2011)	Healthy 20-40 yrs:			Serum IL-6 =, IL-10 =, TNF-α =, TGF-β =. [3]
	Control (56)			
	D3 (47+55+53)	200, 400, 600 IU/d (1,400, 2,800, 4,200)		
	Healthy >64 yrs:			
	Control (54)			
	D3(48+52+48)	200, 400, 600 IU/d (1,400, 2,800, 4,200)	↑ 25(OH)D only for 400 or 600 IU/d	
RCT, PC (3 mts) (Bock *et al.*, 2011)	Healthy:		63.9±27.0 (baseline)	Circulating Treg ↑ [3]
	Control (29)			
	D3 (30)	140,000 IU/ mt (35,000)		

Table 25.2. Continued.

Study			Outcome measures	
Study design (duration)	**Study population (n) + vitamin[1]**	**Dose (with weekly equivalents)**	**Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation)**	**Immunological outcome measures[2]**
RCT, PC (10 wks) (Heine *et al.*, 2011)	Healthy:			Circulating total blood count =, monocytes =
	Control (12)		42.2 to 29.1	Circulating B cell subsets = ##
	D3 (20)	2,000 IU/d (14,000)	28.4 to 80.3	Serum IgA =, IgG =, IgM =, TT-specific IgA =, TT-specific IgG ↑ IgG & IgA = ##, TT-specific plasmablasts =, plasmablasts = ## Circulating TT-specific antibody secreting plasmablasts Supernatants IL-2 =, IL-4 =, IL-5 =, IL-10 =, TNF-α =, IFN-γ = (PBMC+TT/SEB antigen)##. [3]
RCT, PC (1 yr) (Jorde *et al.*, 2010)	Obese:		Baseline values not shown	Serum CRP =, IL-2 =, IL-4 =, IL-5 =, IL-10 =, IL-12 =, IL-13 =, IL-17A =, IFN-γ =, ICAM-1 =, MCP-1 = (# and ##). [4]
	Control (105)		57 (21-111)	
	D3 (98)	20,000 IU/wk	98 (67-176)	
	D3 (104)	40,000 IU/w	141 (40-231)	
RCT, PC (6 wks) (Martineau *et al.*, 2007)	Healthy TB contacts:		35.2 to 67.4	Supernatants IFN-γ = (PBMC+TB antigens) ## *In vitro* anti-mycobacterial potential ↑ at 24h, = at 96h (whole blood+BCG) (# and ##) [5]
	Control (64)			
	D2 (67)	100,000 IU single dose (16,666)		
RCT, PC (3 yrs) (Pittas *et al.*, 2007)	Healthy ≥ 65 yrs:			Serum CRP =, IL-6 = ##. [6]
	Control (161)			
	D3 (153)	700 IU/d (4,900)	76 (baseline) to? (Δ+29.6±3.44/ Δ+31.2±4.4, in 2 subgroups)	

Table 25.2. Continued.

Study			Outcome measures	
Study design (duration)	**Study population (n) + vitamin[1]**	**Dose (with weekly equivalents)**	**Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation)**	**Immunological outcome measures[2]**
OL (4 wks) (Prietl *et al.*, 2010)	Healthy (46) D3	140,000 IU/2 wks (70,000)	Baseline: 59.7±32.2 wk4: 114.6±34.9 wk8: 144.8±37.7	Serum CRP =, circulating Treg ↑. [7]
Autoi-mmune disease				
RCT, PC (1 yr) (Bendix-Struve *et al.*, 2010)	Crohn's disease:			Circulating cell type distribution = *In vitro* proliferaton $CD4^+$ T cells ↑ (anti-CD3/CD28) Supernatants IL-6 ↑##/=#, IL-4 ↑/=, IL-10 =, TNF-a =, IFN-γ = (monocyte-depleted PBMC+anti-CD3/CD28) Circulating Treg =
	Control (10)		61 (22-105) to 46 (27-97) (Δ -5 (-67- 33))	
	D3 (10)	1,200 IU/d (8,400)	33 (16-66) to 118 (62-154) (Δ70 (32-135))	
RCT, OL (52 wks) (Burton *et al.*, 2010; Kimball *et al.*, 2011)	RRMS/SPMS:			Serum CRP =, CTx =, MMP-9 ⁻ (not ##), TIMP-1 ⁻ (not ##), OPN=, BAP=, Kallikrein 6= Serum/ supernatant (PBMC+antigens) IL-1β =, IL-2 =, IL-4 =, IL-5 =, IL-10 =, IL-12p40 =, IL-13 =, TNF-a =, IFNγ = (# and ##) *In vitro* proliferation PBMC (antigens: PHA =, TT=, CS=, BLG=, BSA ⁻, BSAp193 ⁻, BSAp147 =, Tep96 =, GAD =, GADp555 =, PI ⁻, MBP ⁻, EX-2 ⁻, GFAP =, S100β =). [3, 8]
	Control (24)		83 (38-154) to 83±27	
	D3 (25)	4,000-40,000 IU/d (2,800-28,000)	73 (38-146) to 179±27	
RCT, PC (6 mts) (Mahon *et al.*, 2003)	MS:		105.8±37.4 to 174.5±49.9	Serum TGF-b1 ↑ (# and ##) Circulating PBMC mRNA TNF-a =, IFN-γ =, IL-13 =, IL-2 ⁻/=
	Control (22)			
	D3 (17)	1000 IU/d (7,000)		
RCT, PC (6 mts) (Mosayebi *et al.*, 2011)	RRMS:			Supernatant IFN-γ =, TGF-β ↑, IL-10 ↑ ↓ proliferative respons. [9]
	Control (33)		±25 to ±25	
	D3 (26)	300,000 IU/mt (IM) (±75,000)	±25 to ±140	

Table 25.2. Continued.

Study			Outcome measures	
Study design (duration)	**Study population (n) + vitamin[1]**	**Dose (with weekly equivalents)**	**Δ 25(OH)D (nM) (mean or median, pre- to post-supplementation)**	**Immunological outcome measures[2]**
OL (12 wks) (Knippenberg *et al.*, 2011; Smolders *et al.*, 2010b, 2011)	RRMS (15) D3	20,000 IU/d (140,000)	50 (13-175) to 380 (151-535)	Circulating Treg =, Treg suppressive capacity =, $IL10^+CD4^+$ Tcells , $IFN\gamma^+/IL4^+$ $CD4^+$ T cells $^-$. Circulating B cells =, B cell subsets =. Serum IgM =, IgG = & IgA =, BAFF =, OPN =. [3]

[1] Study population in which relevant immunological outcome measures with respect to 25(OH)D levels were measured.

[2] The effect on the outcome measures is given as ↑: increased; ↓: decreased; =: not affected; [#] pre- vs. post-supplementation in intervention group, unless otherwise stated; [##] vitamin D supplementation vs. placebo.

[3] No adverse effects.

[4] = adverse events.

[5] No hypercalcemia or other adverse events.

[6] Well-tolerated.

[7] One mild asymptomatic hypocalcaemia.

[8] Annualized relapse rate $^-$.

[9] No difference in EDSS nor in mean nr. of Gd-enhancing lesions.

Abbreviations: 25(OH)D = 25-dihydroxy vitamin D; BAP = bone-specific alkaline phosphatase; BAFF = B-cell activating factor; BCG = bycobacterium bovis bacille Calmette-Guérin; BLG = β-lactoglobulin; BMD = bone mineral density; BSA = bovine serum albumin; CRP = C-reactive protein; CS = casein; CTx = C-telopeptide; DT = diphtheria toxoid; EX-2 = exon 2 of MBP; GAD = glutamic acid decarboxylase; GFAP = glial fibrillary acidic protein; hCAP = human cathelicidin antimicrobial peptide; IFN = interferon; IL = interleukine; IM = intra muscular; MBP = myelin basic protein; MMP = matrix metalloproteinase; MS = multiple sclerosis; OL = open label; OPN = osteopontin; PHA = phytohemagglutinin; PC = placebo; PI = proinsulin; PPD = purified protein derivative; RCT = randomized controlled trial; RRMS = relapsing remitting multiple sclerosis; SEB = staphylococcal *enterotoxin B;* SI = stimulation index (MTT assay); TB = tubercolosis; Tep = transendothelial protrusion; TGF = tumor growth factor; Th = T-helper; TIMP = tissue inhibitor of metalloproteinase; TLR = toll-like receptor; TNF = tumor necrosis factor; Treg = regulatory T cell; TT = tetanus toxoid; Δ = difference.

no significant differences in serum IL-1β (Kimball *et al.*, 2011), IL-2 (Jorde *et al.*, 2010; Kimball *et al.*, 2011), IL-4 (Jorde *et al.*, 2010; Kimball *et al.*, 2011), IL-5 (Jorde *et al.*, 2010; Kimball *et al.*, 2011), IL-6 (Barnes *et al.*, 2011; Pittas *et al.*, 2007), IL-10 (Barnes *et al.*, 2011; Jorde *et al.*, 2010; Kimball *et al.*, 2011), IL-12 (Jorde *et al.*, 2010; Kimball *et al.*, 2011), IL-13 (Jorde *et al.*, 2010; Kimball *et al.*, 2011), IL-17 (Jorde *et al.*, 2010), IFN-γ (Jorde *et al.*, 2010; Kimball *et al.*, 2011), TGF-β (Barnes *et al.*, 2011), TNF-α (Barnes *et al.*, 2011; Kimball *et al.*, 2011) sICAM-1 (Jorde *et al.*, 2010), MCP-1 (Jorde *et al.*, 2010), C-telopeptide (Kimball *et al.*, 2011), bone-specific alkaline phosphatase (Kimball *et al.*, 2011), kallikrein 6 (Kimball *et al.*, 2011), osteopontin (Kimball *et al.*, 2011; Smolders *et al.*, 2011) or B-cell activating factor (Knippenberg *et al.*, 2011). Despite these negative findings, some studies reported correlations between vitamin D status and soluble molecules in serum. Higher serum levels of 25(OH)D have been reported to coincide with lower serum concentrations of TNF-α in healthy females (Peterson and Heffernan, 2008). In cord blood of newborns, serum 25(OH)D levels correlated positively with levels of IL-10 (Zittermann *et al.*, 2004). This correlation was also observed in females with MCTD (Hajas *et al.*, 2011) and active BD patients (Hamzaoui *et al.*, 2010). Supplementation of vitamin D in patients with MS has been reported to induce increased serum levels of TGF-β (Mahon *et al.*, 2003). Conversely, in otherwise healthy obese females, a negative correlation of serum 25(OH)D with serum IL-5 and IL-10 was reported (Jorde *et al.*, 2010). A negative correlation with IL-6 and IL-23 has been reported in females with MCTD and healthy controls (Hajas *et al.*, 2011; Shea *et al.*, 2008). A long distance holiday induced both higher serum levels of 25(OH)D, sCD14, and sIL-2R (Falkenbach and Sedlmeyer, 1997). MCP-1 was positively correlated to 25(OH)D levels in obese females, but not in males (Jorde *et al.*, 2010). Lastly, in MS patients, lower circulating concentrations of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9 were reported after supplementation of vitamin D (Kimball *et al.*, 2011).

25.6.3 Cytokine excretion by PBMC in supernatants

Of course, the cellular sources of cytokines detected in serum are uncertain. To identify cells within the PBMC fraction producing these cytokines, several groups analyzed the transcription profile of cytokines in PBMC directly *ex vivo*. Supplementation of vitamin D in healthy subjects increased expression of cathelicidin mRNA in monocytes (Adams *et al.*, 2009), but did not differentially affect expression of TNF-α, IFN-γ, IL-2, or IL-13 mRNA in patients with MS (Mahon *et al.*, 2003).

Additionally, several studies measured cytokine excretion in supernatants of specific cell cultures. Most studies cultured PBMC stimulated either with TLR-ligands (Khoo *et al.*, 2011) or with polyclonal (Bendix-Struve *et al.*, 2010) or antigen specific T cell stimuli (Heine *et al.*, 2011; Kimball *et al.*, 2011; Martineau *et al.*, 2007). A higher vitamin D status correlated with a reduced IL-1β and TNF-α production upon TLR2 and TLR4 triggering, while a loss of IL-6, IL-10 and IFN-γ was only found with TLR4 triggering (Khoo *et al.*, 2011). Upon T-cell stimuli, supernatant levels of IL-1β (Kimball *et al.*, 2011), IL-2 (Heine *et al.*, 2011; Kimball *et al.*, 2011), IL-4 (Heine *et al.*, 2011; Kimball *et al.*, 2011), IL-5 (Heine *et al.*, 2011; Kimball *et al.*, 2011), IL-10 (Bendix-Struve *et al.*, 2010; Heine *et al.*, 2011; Kimball *et al.*, 2011), IL-12 (Kimball *et al.*, 2011), IL-13 (Kimball *et*

al., 2011), IFN-γ (Bendix-Struve *et al.*, 2010; Heine *et al.*, 2011; Kimball *et al.*, 2011; Martineau *et al.*, 2007; Mosayebi *et al.*, 2011) and TNF-α (Bendix-Struve *et al.*, 2010; Heine *et al.*, 2011; Kimball *et al.*, 2011), were not affected by supplementation of vitamin D, while TGF-β (Mosayebi *et al.*, 2011) and IL-10 increased (Allen *et al.*, 2012; Mosayebi *et al.*, 2011) and IL-17 decreased (Allen *et al.*, 2012). The positive effect on IL-10 was mainly due to an increased production of IL-10 by the $CD4^+$ depleted PBMCs, while this cell population did not produce IL-17 (Allen *et al.*, 2012). A study on polyclonal stimulation of monocyte-depleted PBMC of patients with Crohn's disease reported a trend towards an increased concentration of IL-4 and IL-6 in the supernatants after supplementation (Bendix-Struve *et al.*, 2010).

25.6.4 Proliferation of T cells *in vitro*

Another way to measure T cell responses to antigens, is to measure induction of proliferation *in vitro*. A study in Crohn's disease patients reported an increase of polyclonal T cell proliferation after supplementation of vitamin D (Bendix-Struve *et al.*, 2010), while in MS patients proliferation was decreased (Mosayebi *et al.*, 2011) or unaffected (Smolders *et al.*, 2010a). Kimball *et al.* (2011) tested proliferative responses of T cells against a large panel of antigens in MS patients. After supplementation of vitamin D, proliferative responses against BSA, BSAp193, proinsulin, MBP and exon 2 of MBP were reduced compared with prior to supplementation.

25.6.5 Phenotyping PBMC directly *ex vivo*

Several studies analyzed circulating myeloid and lymphocyte subsets and phenotypes with flowcytometry. Two studies reported a negative correlation between serum 25(OH)D levels and monocyte expression levels of both TLR-2 (Do *et al.*, 2008; Khoo *et al.*, 2011) and TLR-4 (Do *et al.*, 2008; Khoo *et al.*, 2011). There was neither a correlation with the circulating proportions of myeloid and plasmacytoid DCs, nor with the expression of human leukocyte antigen-DR, CD40, or CD86 on these cells (Ben-Zvi *et al.*, 2010). There was also no correlation with the proportion of $CD14^+CD16^+$ monocytes (Do *et al.*, 2008). Vitamin D supplementation did not affect the total proportion of circulating monocytes (Heine *et al.*, 2011).

Regarding lymphocytes, both B and T cell subsets have been characterized. A correlation was described of 25(OH)D levels with the percentages of total naïve ($CXCR3^+$) and total memory ($CXCR^+$) $CD4^+$ T cells in an MS cohort not treated with immune modulating therapies (Royal *et al.*, 2009). In patients with BD, a positive correlation between 25(OH)D and total proportions of Treg was reported (Hamzaoui *et al.*, 2010). Such a correlation was not confirmed in patients with MS (Smolders *et al.*, 2010a), and was even contradicted by a negative correlation in another MS population (Royal *et al.*, 2009). When assessing subsets of Tregs, lower circulating proportions of $CD39^+$ Treg correlated with higher serum 25(OH)D levels in MS patients during disease remission (Peelen *et al.*, 2011a). Additionally, the capacity of Treg to control polyclonal T cell proliferation was also improved in MS patients with higher serum 25(OH)D levels (Smolders *et al.*, 2009). Supplementation of vitamin D has been reported to induce higher circulating proportions of Treg in healthy individuals (Bock *et al.*, 2011; Prietl *et al.*, 2010). Contrastingly, supplementation of

vitamin D did not result in higher proportions of naïve/ memory Treg in subjects with MS and Crohn's disease (Bendix-Struve *et al.*, 2010; Smolders *et al.*, 2010b), and Treg suppressive capacity was also not significantly affected (Smolders *et al.*, 2010b). Regarding T helper cell subsets, a study in BD patients reported negative correlations between serum 25(OH)D and the circulating proportion of Th1 and Th17 cells, and with the Th1/Th2-ratio (Hamzaoui *et al.*, 2010). Although a study in MS patients could not detect correlations with the proportions of T helper cell subsets, the Th1/Th2 ratio correlated also negatively with serum 25(OH)D (Smolders *et al.*, 2009). In a cross-sectional study among MS patients, serum 25(OH)D levels tended to be lower in patients with an expanded Th17 compartment (Peelen *et al.*, 2011a). Supplementation of vitamin D in healthy subjects resulted in a decline of Th17 cells, while the number of IL-10^+ T cells did not change (Allen *et al.*, 2012). Supplementation of vitamin D in subjects with MS resulted in a decreased Th1/Th2-ratio, as well as an expanded proportion of IL-10^+ T cells (Smolders *et al.*, 2010b).

With respect to B cells, a study in SLE reported decreased B cell activation in subjects with high 25(OH)D levels (Ritterhouse *et al.*, 2011). However, in subjects with MS, no correlation of serum 25(OH)D with circulating B cell subsets, including regulatory B cells, could be found (Knippenberg *et al.*, 2011). Accordingly, supplementation of vitamin D did not affect B cell subset distribution in both subjects with MS and healthy control donors (Heine *et al.*, 2011; Knippenberg *et al.*, 2011). B cells are precursors to Ig-producing plasma cells. A study in SLE and in healthy individuals on long distance holiday reported lower total IgG concentrations in serum with increasing 25(OH)D level (Falkenbach and Sedlmeyer, 1997; Szodoray *et al.*, 2011), which might be in line with the reduced B cell activation observed in the cross-sectional study (Ritterhouse *et al.*, 2011). IgE production seems to be affected negatively by 25(OH)D (Zittermann *et al.*, 2004), while IgA production was increased and IgM production was not correlated (Falkenbach and Sedlmeyer, 1997). On the other hand, other studies found no effect of vitamin D supplementation on total serum polyclonal IgG, IgM and IgA in healthy subjects and subjects with MS (Heine *et al.*, 2011; Knippenberg *et al.*, 2011). However, antigen specific antibody-production may be more relevant than the polyclonal compartment. Indeed, supplementation of vitamin D improved tetanus toxoid-specific IgG-response in tetanus toxoid vaccination (Heine *et al.*, 2011). In several SLE cohorts contrasting results have been found regarding antinuclear antibody and anti-double stranded DNA antibody production (Bonakdar *et al.*, 2011; Ritterhouse *et al.*, 2011; Szodoray *et al.*, 2011; Thudi *et al.*, 2008). A negative correlation for serum 25(OH)D with antithyroid antibodies in thyroid disease patients (Kivity *et al.*, 2011), with serum anti-human tissue transglutaminase IgA in ankylosing spondylitis and psoriatric arthritis patients (Teichmann *et al.*, 2010) and a trend towards such a correlation with anti-SS-A-antibodies in SLE (Ritterhouse *et al.*, 2011) have been reported, while there was a positive correlation with serum anti-Smith antibodies (Szodoray *et al.*, 2011). In donors at risk for rheumatoid arthritis, there was no correlation of 25(OH)D with either rheumatoid factor or anti-cyclic citrullinated peptide antibodies (Feser *et al.*, 2009). In patients with MCTD, serum anti-U1-ribonucleoprotein-antibody correlated negatively and serum anti-CL IgA positively with serum 25(OH)D, while no correlation with anti-CL IgG/IgM and anti-endothelial cell antibodies was found (Hajas *et al.*, 2011).

25.7 Discussion and conclusion

Our literature search revealed evidently that studies done so far are heterogeneous in many aspects. Study populations range from healthy controls to at risk persons and patients with distinct auto-immune diseases. Moreover, most studies seem to be underpowered and have a lack of appropriate controls. Beside this, 25(OH)D status is not always properly reported. This is regrettable, since an effect of vitamin D on the immune system would be expected in the highest or higher than physiological ranges of 25(OH)D levels (Baeke *et al.*, 2010). Without baseline 25(OH)D levels, it is difficult to discriminate between the effects of vitamin D in individuals with sufficient versus insufficient vitamin D status. Besides this, 25(OH)D assays have a great interassay variability (Lai *et al.*, 2012) and cut-off points used for defining 25(OH)D insufficiency and sufficiency are not standardized. Furthermore, correlation studies have the potential bias of reverse causality: vitamin D status might not be responsible for the possible improvement in clinical and immunological outcomes in disease, but disability itself might influence the vitamin D status of individuals. Supplementation studies are not subjected to reverse causality, however, these also show great diversity in the supplemented compound (either vitamin D2 or vitamin D3) and in the dose and duration of supplementation. The supplemented doses range from doses which do not affect serum 25(OH)D levels significantly to doses which lead to supra-physiological serum 25(OH)D levels. These factors make the individual studies hardly comparable, and readily explains the many contradictive results. Finally, it is also important to keep a publication bias in consideration. In general, data suggest a trend towards a promotion of anti-inflammatory/regulatory cytokines and cells to be present, but this is certainly not substantiated by conclusive data.

On top of that, analyses of the studies thus far showed a diverse repertoire of immunological outcome measures. These outcome measures form a continuum, ranging from soluble markers of general inflammation which can be measured easily in large cohorts of study subjects, to labour-intensive, detailed assessment of specific cellular processes. We argue that the choice between either of these outcome measures should primarily be driven by the specific disease outcome that is to be studied. As described above, $1,25(OH)_2D$ interferes *in vitro* with proliferation and cytokine secretion by activated PBMC. These *in vitro* models reflect most likely the sites in the body where antigen presentation and immune activation take place, which are the secondary lymphoid organs and the tissues targeted by auto-immune diseases. In these tissues, the interaction of vitamin D with the immune response is unlikely to depend on either a single cell type or cytokine, but rather on a complex interplay between multiple vitamin D sensitive cells of the innate and adaptive immune system (Peelen *et al.*, 2011b). When analyzing correlations between serum 25(OH)D levels and immunological parameters in the blood, one has to realize that there is a mismatch in space and time related to *in vitro* experiments. Immune cells in the circulation are on the move between target sites and lymphoid tissue, and soluble factors are derived from both specific immune responses of interest as well as from other cells. Most informative would be assessment of cells and cytokines in lymph nodes and target organs, but sampling of material is obviously not feasible in most diseases. Another issue to keep in mind is that effects of vitamin D on immune parameters, as described in this chapter, might be secondary to other effects of

vitamin D. For instance, the positive correlation between 25(OH)D and C4 in SLE might be due to an immune independent effect on disease activity; since SLE is an immune complex mediated disease associated with complement activation via the classical pathway, any reduction in disease activity will result in normalization of C4 levels. Similarly, the negative association of 25(OH)D levels with several auto-antibodies might not be due to a direct effect of vitamin D on B cells, but might be mediated via an effect on auto-antigen presentation by APCs. In addition, many auto-immune diseases are associated with infections (Kivity *et al.*, 2009) causing inflammation and subsequent induction of auto-immune disease manifestations. Since vitamin D also affects the innate immune system via the production of beta-defensins and cathelicidins, this will reduce the infectious load and thereby the inflammatory response and eventually will affect the adaptive immune system. To substantiate an immune modulating effect of vitamin D *in vivo*, the best approach appears to be assessment of the endproducts of the immunological cascade in the circulation, which are most relevant to the disease or health outcome studied. For auto-immune diseases, analysis of auto-antibody titers or lymphocyte cell proliferative and cytokine responses against auto-antigens are more logical choices than assessment of polyclonal IgG levels, polyclonal T cell proliferation, or serum cytokine levels. In MS, where no single auto-antigen is present, studies in which immunological outcomes have been associated with disease in patient-control studies, or have been associated with exacerbation activity or remission of disease, seem most relevant. Ultimately, only well-powered, controlled studies in well defined cohorts measuring disease-specific relevant outcome measures will be most informative on a immune modulating potential of vitamin D.

Currently, several clinical trials are investigating the efficacy of vitamin D supplementation in MS and other auto-immune diseases. These trials provide an excellent framework to design potentially conclusive studies on the immune-modulating effects of vitamin D *in vivo*. It would be a missed chance not to address this issue in these studies, especially since insight in the working mechanism of vitamin D may reveal subgroups of patients which may benefit most from vitamin D supplementation. Additionally, a known working mechanism can facilitate hypothesis driven combination with existing treatment regimens, and may reveal a biomarker which allows titration of serum 25(OH)D levels to an adequate dose.

References

Adams, J.S., Ren, S., Liu, P.T., Chun, R.F., Lagishetty, V., Gombart, A.F., Borregaard, N., Modlin, R.L. and Hewison, M., 2009. Vitamin d-directed rheostatic regulation of monocyte antibacterial responses. Journal of Immunology 182, 4289-4295.

Allen, A.C., Kelly, S., Basdeo, S.A., Kinsella, K., Mulready, K.J., Mills, K.H., Tubridy, N., Walsh, C., Brady, J.J., Hutchinson, M. and Fletcher, J.M., 2012. A pilot study of the immunological effects of high-dose vitamin D in healthy volunteers. Multiple Sclerosis 18, 1797-1800.

Baeke, F., Takiishi, T., Korf, H., Gysemans, C. and Mathieu, C., 2010. Vitamin D: modulator of the immune system. Current Opinion in Pharmacology 10, 482-496.

Bar-Or, A., 2008. The immunology of multiple sclerosis. Seminars in Neurology 28, 29-45.

Barnes, M.S., Horigan, G., Cashman, K.D., Hill, T.R., Forsythe, L.K., Lucey, A.J., McSorley, E.M., Kiely, M., Bonham, M.P., Magee, P.J., Strain, J.J. and Wallace, J.M., 2011. Maintenance of wintertime vitamin D status with cholecalciferol supplementation is not associated with alterations in serum cytokine concentrations among apparently healthy younger or older adults. Journal of Nutrition 141, 476-481.

Bendix-Struve, M., Bartels, L.E., Agnholt, J., Dige, A., Jorgensen, S.P. and Dahlerup, J.F., 2010. Vitamin D3 treatment of Crohn's disease patients increases stimulated T cell IL-6 production and proliferation. Alimentary Pharmacology and Therapeutics 32, 1364-1372.

Ben-Zvi, I., Aranow, C., Mackay, M., Stanevsky, A., Kamen, D.L., Marinescu, L.M., Collins, C.E., Gilkeson, G.S., Diamond, B. and Hardin, J.A., 2010. The impact of vitamin D on dendritic cell function in patients with systemic lupus erythematosus. PLoS One 5, e9193.

Bock, G., Prietl, B., Mader, J.K., Holler, E., Wolf, M., Pilz, S., Graninger, W.B., Obermayer-Pietsch, B.M. and Pieber, T.R., 2011. The effect of vitamin D supplementation on peripheral regulatory T cells and beta cell function in healthy humans: a randomized controlled trial. Diabetes/Metabolism Research and Reviews 27, 942-945.

Bonakdar, Z.S., Jahanshahifar, L., Jahanshahifar, F. and Gholamrezaei, A., 2011. Vitamin D deficiency and its association with disease activity in new cases of systemic lupus erythematosus. Lupus 20, 1155-1160.

Borsellino, G., Kleinewietfeld, M., Di Mitri, D., Sternjak, A., Diamantini, A., Giometto, R., Hopner, S., Centonze, D., Bernardi, G., Dell'Acqua, M.L., Rossini, P.M., Battistini, L., Rotzschke, O. and Falk, K., 2007. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood 110, 1225-1232.

Burton, J.M., Kimball, S., Vieth, R., Bar-Or, A., Dosch, H.M., Cheung, R., Gagne, D., D'Souza, C., Ursell, M. and O'Connor, P., 2010. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. Neurology 74, 1852-1859.

Cohen, J.A., Barkhof, F., Comi, G., Hartung, H.P., Khatri, B.O., Montalban, X., Pelletier, J., Capra, R., Gallo, P., Izquierdo, G., Tiel-Wilck, K., de Vera, A., Jin, J., Stites, T., Wu, S., Aradhye, S. and Kappos, L., 2010. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. New England Journal of Medicine 362, 402-415.

Coles, A.J., Compston, D.A., Selmaj, K.W., Lake, S.L., Moran, S., Margolin, D.H., Norris, K. and Tandon, P.K., 2008. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. New England Journal of Medicine 359, 1786-1801.

Compston, A. and Coles, A., 2002. Multiple sclerosis. Lancet 359, 1221-1231.

Compston, A. and Coles, A., 2008. Multiple sclerosis. Lancet 372, 1502-1517.

Correale, J., Ysrraelit, M.C. and Gaitan, M.I., 2009. Immunomodulatory effects of Vitamin D in multiple sclerosis. Brain 132, 1146-1160.

Dalla Libera, D., Di Mitri, D., Bergami, A., Centonze, D., Gasperini, C., Grasso, M.G., Galgani, S., Martinelli, V., Comi, G., Avolio, C., Martino, G., Borsellino, G., Sallusto, F., Battistini, L. and Furlan, R., 2011. T regulatory cells are markers of disease activity in multiple sclerosis patients. PLoS One 6, e21386.

Do, J.E., Kwon, S.Y., Park, S. and Lee, E.S., 2008. Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behcet's disease. Rheumatology 47, 840-848.

Durelli, L., Conti, L., Clerico, M., Boselli, D., Contessa, G., Ripellino, P., Ferrero, B., Eid, P. and Novelli, F., 2009. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. Annals of Neurology 65, 499-509.

Dusso, A.S., Brown, A.J. and Slatopolsky, E., 2005. Vitamin D. American Journal of Physiology: Renal Physiology 289, F8-28.

Edstrom, M., Mellergard, J., Mjosberg, J., Jenmalm, M., Vrethem, M., Press, R., Dahle, C. and Ernerudh, J., 2011. Transcriptional characteristics of CD4+ T cells in multiple sclerosis: relative lack of suppressive populations in blood. Multiple Sclerosis 17, 57-66.

Edwards, L.J., Robins, R.A. and Constantinescu, C.S., 2010. Th17/Th1 phenotype in demyelinating disease. Cytokine 50, 19-23.

Falkenbach, A. and Sedlmeyer, A., 1997. Travel to sunny countries is associated with changes in immunological parameters. Photodermatology, Photoimmunology and Photomedicine 13, 139-142.

Feser, M., Derber, L.A., Deane, K.D., Lezotte, D.C., Weisman, M.H., Buckner, J.H., Mikuls, T., O'Dell, J., Gregersen, P.K., Holers, V.M. and Norris, J.M., 2009. Plasma 25,OH vitamin D concentrations are not associated with rheumatoid arthritis (RA)-related autoantibodies in individuals at elevated risk for RA. Journal of Rheumatology 36, 943-946.

Fletcher, J.M., Lonergan, R., Costelloe, L., Kinsella, K., Moran, B., O'Farrelly, C., Tubridy, N. and Mills, K.H., 2009. CD39+Foxp3+ regulatory T Cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. Journal of Immunology 183, 7602-7610.

Frischer, J.M., Bramow, S., Dal-Bianco, A., Lucchinetti, C.F., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, P.S. and Lassmann, H., 2009. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 132, 1175-1189.

Frisullo, G., Nociti, V., Iorio, R., Patanella, A.K., Marti, A., Caggiula, M., Mirabella, M., Tonali, P.A. and Batocchi, A.P., 2008. IL17 and IFNgamma production by peripheral blood mononuclear cells from clinically isolated syndrome to secondary progressive multiple sclerosis. Cytokine 44, 22-25.

Gottfried, E., Rehli, M., Hahn, J., Holler, E., Andreesen, R. and Kreutz, M., 2006. Monocyte-derived cells express CYP27A1 and convert vitamin D3 into its active metabolite. Biochemical and Biophysical Research Communications 349, 209-213.

Haas, J., Hug, A., Viehover, A., Fritzsching, B., Falk, C.S., Filser, A., Vetter, T., Milkova, L., Korporal, M., Fritz, B., Storch-Hagenlocher, B., Krammer, P.H., Suri-Payer, E. and Wildemann, B., 2005. Reduced suppressive effect of CD4+CD25high regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. European Journal of Immunology 35, 3343-3352.

Hajas, A., Sandor, J., Csathy, L., Csipo, I., Barath, S., Paragh, G., Seres, I., Szegedi, G., Shoenfeld, Y. and Bodolay, E., 2011. Vitamin D insufficiency in a large MCTD population. Autoimmunity Reviews 10, 317-324.

Hamzaoui, K., Ben Dhifallah, I., Karray, E., Sassi, F.H. and Hamzaoui, A., 2010. Vitamin D modulates peripheral immunity in patients with Behcet's disease. Clinical and Experimental Rheumatology 28, S50-57.

Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., Bar-Or, A., Panzara, M., Sarkar, N., Agarwal, S., Langer-Gould, A. and Smith, C.H., 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. New England Journal of Medicine 358, 676-688.

Hayes, C.E., Cantorna, M.T. and DeLuca, H.F., 1997. Vitamin D and multiple sclerosis. Proceedings of the Society for Experimental Biology and Medicine 216, 21-27.

Heine, G., Drozdenko, G., Lahl, A., Unterwalder, N., Mei, H., Volk, H.D., Dorner, T., Radbruch, A. and Worm, M., 2011. Efficient tetanus toxoid immunization on vitamin D supplementation. European Journal of Clinical Nutrition 65, 329-334.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Jongen, M.J., van der Vijgh, W.J., Lips, P. and Netelenbos, J.C., 1984. Measurement of vitamin D metabolites in anephric subjects. Nephron 36, 230-234.

Jorde, R., Sneve, M., Torjesen, P.A., Figenschau, Y., Goransson, L.G. and Omdal, R., 2010. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine 50, 175-180.

Junker, A., Ivanidze, J., Malotka, J., Eiglmeier, I., Lassmann, H., Wekerle, H., Meinl, E., Hohlfeld, R. and Dornmair, K., 2007. Multiple sclerosis: T-cell receptor expression in distinct brain regions. Brain 130, 2789-2799.

Khoo, A.L., Chai, L.Y., Koenen, H.J., Sweep, F.C., Joosten, I., Netea, M.G. and van der Ven, A.J., 2011. Regulation of cytokine responses by seasonality of vitamin D status in healthy individuals. Clinical and Experimental Immunology 164, 72-79.

Kimball, S.M., Ursell, M.R., O'Connor, P. and Vieth, R., 2007. Safety of vitamin D3 in adults with multiple sclerosis. American Journal of Clinical Nutriton 86, 645-651.

Kimball, S., Vieth, R., Dosch, H.M., Bar-Or, A., Cheung, R., Gagne, D., O'Connor, P., D'Souza, C., Ursell, M. and Burton, J.M., 2011. Cholecalciferol plus calcium suppresses abnormal PBMC reactivity in patients with multiple sclerosis. Journal of Clinical Endocrinology and Metabolism 96, 2826-2834.

Kivity, S., Agmon-Levin, N., Blank, M. and Shoenfeld, Y., 2009. Infections and autoimmunity – friends or foes? Trends in Immunology 30, 409-414.

Kivity, S., Agmon-Levin, N., Zisappl, M., Shapira, Y., Nagy, E.V., Danko, K., Szekanecz, Z., Langevitz, P. and Shoenfeld, Y., 2011. Vitamin D and autoimmune thyroid diseases. Cellular and Molecular Immunology 8, 243-247.

Knippenberg, S., Smolders, J., Thewissen, M., Peelen, E., Tervaert, J.W., Hupperts, R. and Damoiseaux, J., 2011. Effect of vitamin D(3) supplementation on peripheral B cell differentiation and isotype switching in patients with multiple sclerosis. Multiple Sclerosis 17, 1418-1423.

Lai, J.K., Lucas, R.M., Banks, E. and Ponsonby, A.L., 2012. Variability in vitamin D assays impairs clinical assessment of vitamin D status. Internal Medicine Journal 42, 43-50.

Lips, P., 2006. Vitamin D physiology. Progress in Biophysics and Molecular Biology 92, 4-8.

Mahon, B.D., Gordon, S.A., Cruz, J., Cosman, F. and Cantorna, M.T., 2003. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. Journal of Neuroimmunology 134, 128-132.

Martineau, A.R., Wilkinson, R.J., Wilkinson, K.A., Newton, S.M., Kampmann, B., Hall, B.M., Packe, G.E., Davidson, R.N., Eldridge, S.M., Maunsell, Z.J., Rainbow, S.J., Berry, J.L. and Griffiths, C.J., 2007. A single dose of vitamin D enhances immunity to mycobacteria. American Journal of Respiratory and Critical Care Medicine 176, 208-213.

Matusevicius, D., Kivisakk, P., He, B., Kostulas, N., Ozenci, V., Fredrikson, S. and Link, H., 1999. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Multiple Sclerosis 5, 101-104.

Milo, R. and Kahana, E., 2010. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmunity Reviews 9, A387-394.

Mosayebi, G., Ghazavi, A., Ghasami, K., Jand, Y. and Kokhaei, P., 2011. Therapeutic effect of vitamin D3 in multiple sclerosis patients. Immunological Investigations 40, 627-639.

Mowry, E.M., Krupp, L.B., Milazzo, M., Chabas, D., Strober, J.B., Belman, A.L., McDonald, J.C., Oksenberg, J.R., Bacchetti, P. and Waubant, E., 2010. Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis. Annals of Neurology 67, 618-624.

Munger, K.L., Levin, L.I., Hollis, B.W., Howard, N.S. and Ascherio, A., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296, 2832-2838.

Noronha, A., Toscas, A. and Jensen, M.A., 1993. Interferon beta decreases T cell activation and interferon gamma production in multiple sclerosis. Journal of Neuroimmunology 46, 145-153.

Peelen, E., Damoiseaux, J., Smolders, J., Knippenberg, S., Menheere, P., Tervaert, J.W., Hupperts, R. and Thewissen, M., 2011a. Th17 expansion in MS patients is counterbalanced by an expanded CD39+ regulatory T cell population during remission but not during relapse. Journal of Neuroimmunology 240-241, 97-103.

Peelen, E., Knippenberg, S., Muris, A.H., Thewissen, M., Smolders, J., Tervaert, J.W., Hupperts, R. and Damoiseaux, J., 2011b. Effects of vitamin D on the peripheral adaptive immune system: a review. Autoimmunity Reviews 10, 733-743.

Penna, G., Amuchastegui, S., Giarratana, N., Daniel, K.C., Vulcano, M., Sozzani, S. and Adorini, L., 2007. 1,25-Dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. Journal of Immunology 178, 145-153.

Peterson, C.A. and Heffernan, M.E., 2008. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond) 5, 10.

Pittas, A.G., Harris, S.S., Stark, P.C. and Dawson-Hughes, B., 2007. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 30, 980-986.

Polman, C.H., O'Connor, P.W., Havrdova, E., Hutchinson, M., Kappos, L., Miller, D.H., Phillips, J.T., Lublin, F.D., Giovannoni, G., Wajgt, A., Toal, M., Lynn, F., Panzara, M.A. and Sandrock, A.W., 2006. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. New England Journal of Medicine 354, 899-910.

Prietl, B., Pilz, S., Wolf, M., Tomaschitz, A., Obermayer-Pietsch, B., Graninger, W. and Pieber, T.R., 2010. Vitamin D supplementation and regulatory T cells in apparently healthy subjects: vitamin D treatment for autoimmune diseases? Israel Medical Association Journal 12, 136-139.

Quintana, F.J., Farez, M.F., Izquierdo, G., Lucas, M., Cohen, I.R. and Weiner, H.L., 2012. Antigen microarrays identify CNS-produced autoantibodies in RRMS. Neurology 78, 532-539.

Ritterhouse, L.L., Crowe, S.R., Niewold, T.B., Kamen, D.L., Macwana, S.R., Roberts, V.C., Dedeke, A.B., Harley, J.B., Scofield, R.H., Guthridge, J.M. and James, J.A., 2011. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. Annals of the Rheumatic Diseases 70, 1569-1574.

Rosati, G., 2001. The prevalence of multiple sclerosis in the world: an update. Neurological Sciences 22, 117-139.

Royal, W., 3rd, Mia, Y., Li, H. and Naunton, K., 2009. Peripheral blood regulatory T cell measurements correlate with serum vitamin D levels in patients with multiple sclerosis. Journal of Neuroimmunology 213, 135-141.

Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S.E., Edkins, S., Gray, E., Booth, D.R., Potter, S.C., Goris, A., Band, G., Oturai, A.B., Strange, A., Saarela, J., Bellenguez, C., Fontaine, B., Gillman, M., Hemmer, B., Gwilliam, R., Zipp, F., Jayakumar, A., Martin, R., Leslie, S., Hawkins, S., Giannoulatou, E., D'Alfonso, S., Blackburn, H., Boneschi, F.M., Liddle, J., Harbo, H.F., Perez, M.L., Spurkland, A., Waller, M.J., Mycko, M.P., Ricketts, M., Comabella, M., Hammond, N., Kockum, I., McCann, O.T., Ban, M., Whittaker, P., Kemppinen, A., Weston, P., Hawkins, C., Widaa, S., Zajicek, J., Dronov, S., Robertson, N., Bumpstead, S.J., Barcellos, L.F., Ravindrarajah, R., Abraham, R., Alfredsson, L., Ardlie, K., Aubin, C., Baker, A., Baker, K., Baranzini, S.E., Bergamaschi, L., Bergamaschi, R., Bernstein, A., Berthele, A., Boggild, M., Bradfield, J.P., Brassat, D., Broadley, S.A., Buck, D., Butzkueven, H., Capra, R., Carroll, W.M., Cavalla, P., Celius, E.G., Cepok, S., Chiavacci, R., Clerget-Darpoux, F., Clysters, K., Comi, G., Cossburn, M., Cournu-Rebeix, I., Cox, M.B., Cozen, W., Cree, B.A., Cross, A.H., Cusi, D., Daly, M.J., Davis, E., de Bakker, P.I., Debouverie, M., D'Hooghe M, B., Dixon, K., Dobosi, R., Dubois, B., Ellinghaus, D., Elovaara, I., Esposito, F.,

Fontenille, C., Foote, S., Franke, A., Galimberti, D., Ghezzi, A., Glessner, J., Gomez, R., Gout, O., Graham, C., Grant, S.F., Guerini, F.R., Hakonarson, H., Hall, P., Hamsten, A., Hartung, H.P., Heard, R.N., Heath, S., Hobart, J., Hoshi, M., Infante-Duarte, C., Ingram, G., Ingram, W., Islam, T., Jagodic, M., Kabesch, M., Kermode, A.G., Kilpatrick, T.J., Kim, C., Klopp, N., Koivisto, K., Larsson, M., Lathrop, M., Lechner-Scott, J.S., Leone, M.A., Leppa, V., Liljedahl, U., Bomfim, I.L., Lincoln, R.R., Link, J., Liu, J., Lorentzen, A.R., Lupoli, S., Macciardi, F., Mack, T., Marriott, M., Martinelli, V., Mason, D., McCauley, J.L., Mentch, F., Mero, I.L., Mihalova, T., Montalban, X., Mottershead, J., Myhr, K.M., Naldi, P., Ollier, W., Page, A., Palotie, A., Pelletier, J., Piccio, L., Pickersgill, T., Piehl, F., Pobywajlo, S., Quach, H.L., Ramsay, P.P., Reunanen, M., Reynolds, R., Rioux, J.D., Rodegher, M., Roesner, S., Rubio, J.P., Ruckert, I.M., Salvetti, M., Salvi, E., Santaniello, A., Schaefer, C.A., Schreiber, S., Schulze, C., Scott, R.J., Sellebjerg, F., Selmaj, K.W., Sexton, D., Shen, L., Simms-Acuna, B., Skidmore, S., Sleiman, P.M., Smestad, C., Sorensen, P.S., Sondergaard, H.B., Stankovich, J., Strange, R.C., Sulonen, A.M., Sundqvist, E., Syvanen, A.C., Taddeo, F., Taylor, B., Blackwell, J.M., Tienari, P., Bramon, E., Tourbah, A., Brown, M.A., Tronczynska, E., Casas, J.P., Tubridy, N., Corvin, A., Vickery, J., Jankowski, J., Villoslada, P., Markus, H.S., Wang, K., Mathew, C.G., Wason, J., Palmer, C.N., Wichmann, H.E., Plomin, R., Willoughby, E., Rautanen, A., Winkelmann, J., Wittig, M., Trembath, R.C., Yaouanq, J., Viswanathan, A.C., Zhang, H., Wood, N.W., Zuvich, R., Deloukas, P., Langford, C., Duncanson, A., Oksenberg, J.R., Pericak-Vance, M.A., Haines, J.L., Olsson, T., Hillert, J., Ivinson, A.J., De Jager, P.L., Peltonen, L., Stewart, G.J., Hafler, D.A., Hauser, S.L., McVean, G., Donnelly, P. and Compston, A., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 476, 214-219.

Shea, M.K., Booth, S.L., Massaro, J.M., Jacques, P.F., D'Agostino, R.B., Sr., Dawson-Hughes, B., Ordovas, J.M., O'Donnell, C.J., Kathiresan, S., Keaney Jr., J.F., Vasan, R.S. and Benjamin, E.J., 2008. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. American Journal of Epidemiology 167, 313-320.

Simpson Jr., S., Taylor, B., Blizzard, L., Ponsonby, A.L., Pittas, F., Tremlett, H., Dwyer, T., Gies, P. and van der Mei, I., 2010. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. Annals of Neurology 68, 193-203.

Smolders, J. and Damoiseaux, J., 2011. Vitamin D as a T-cell modulator in multiple sclerosis. Vitamins and Hormones 86, 401-428.

Smolders, J., Menheere, P., Kessels, A., Damoiseaux, J. and Hupperts, R., 2008. Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis. Multiple Sclerosis 14, 1220-1224.

Smolders, J., Menheere, P., Thewissen, M., Peelen, E., Tervaert, J.W., Hupperts, R. and Damoiseaux, J., 2010a. Regulatory T cell function correlates with serum 25-hydroxyvitamin D, but not with 1,25-dihydroxyvitamin D, parathyroid hormone and calcium levels in patients with relapsing remitting multiple sclerosis. Journal of Steroid Biochemistry and Molecular Biology 121, 243-246.

Smolders, J., Peelen, E., Thewissen, M., Cohen Tervaert, J.W., Menheere, P., Hupperts, R. and Damoiseaux, J., 2010b. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. PloS One 5, e15235.

Smolders, J., Peelen, E., Thewissen, M., Menheere, P., Tervaert, J.W., Hupperts, R. and Damoiseaux, J., 2009a. The relevance of vitamin D receptor gene polymorphisms for vitamin D research in multiple sclerosis. Autoimmunity Reviews 8, 621-626.

Smolders, J., Thewissen, M., Peelen, E., Menheere, P., Tervaert, J.W., Damoiseaux, J. and Hupperts, R., 2009. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. PloS One 4, e6635.

Smolders, J., Vogt, M. and Hupperts, R., 2011. Vitamin D3 supplementation does not affect circulating osteopontin levels in MS. Journal of Clinical Endocrinology and Metabolism (eLetter).

Spach, K.M. and Hayes, C.E., 2005. Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. Journal of Immunology 175, 4119-4126.

Szodoray, P., Tarr, T., Bazso, A., Poor, G., Szegedi, G. and Kiss, E., 2011. The immunopathological role of vitamin D in patients with SLE: data from a single centre registry in Hungary. Scandinavian Journal of Rheumatology 40, 122-126.

Teichmann, J., Voglau, M.J. and Lange, U., 2010. Antibodies to human tissue transglutaminase and alterations of vitamin D metabolism in ankylosing spondylitis and psoriatic arthritis. Rheumatology International 30, 1559-1563.

Thudi, A., Yin, S., Wandstrat, A.E., Li, Q.Z. and Olsen, N.J., 2008. Vitamin D levels and disease status in Texas patients with systemic lupus erythematosus. American Journal of the Medical Sciences 335, 99-104.

Venken, K., Hellings, N., Hensen, K., Rummens, J.L., Medaer, R., D'Hooghe M, B., Dubois, B., Raus, J. and Stinissen, P., 2006. Secondary progressive in contrast to relapsing-remitting multiple sclerosis patients show a normal CD4+CD25+ regulatory T-cell function and FOXP3 expression. Journal of Neuroscience Research 83, 1432-1446.

Viglietta, V., Baecher-Allan, C., Weiner, H.L. and Hafler, D.A., 2004. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. Journal of Experimental Medicine 199, 971-979.

Vilarrasa, N., Vendrell, J., Maravall, J., Elio, I., Solano, E., San Jose, P., Garcia, I., Virgili, N., Soler, J. and Gomez, J.M., 2010. Is plasma 25(OH) D related to adipokines, inflammatory cytokines and insulin resistance in both a healthy and morbidly obese population? Endocrine 38, 235-242.

Wingerchuk, D.M., Lesaux, J., Rice, G.P., Kremenchutzky, M. and Ebers, G.C., 2005. A pilot study of oral calcitriol (1,25-dihydroxyvitamin D3) for relapsing-remitting multiple sclerosis. Journal of Neurology, Neurosurgery and Psychiatry 76, 1294-1296.

Zittermann, A., Dembinski, J. and Stehle, P., 2004. Low vitamin D status is associated with low cord blood levels of the immunosuppressive cytokine interleukin-10. Pediatric Allergy and Immunology 15, 242-246.

Vitamin D
in chronic diseases

Key facts

- Insulin resistance often presents with hyperglycemia despite elevated serum levels of insulin.
- Prolonged hypersecretion of insulin, as seen in insulin-resistant states, may lead to loss of pancreatic β-cell function and the eventual development of type 2 diabetes.
- Skeletal muscle is the primary site of glucose disposal, and therefore plays a key role in regulating whole-body glucose homeostasis.
- 25(OH)D 1α-hydroxylase is the enzyme responsible for converting 25-hydroxyvitamin D (25(OH)D), the precursor to the biologically active form, to 1α,25$(OH)_2$D, the hormonally active form of vitamin D.
- 1α,25$(OH)_2$D3, the active form of vitamin D, induces genomic responses through interaction with the nuclear vitamin D receptor, as well as non-transcriptional responses involving activation of transmembrane signal transduction pathways via a putative novel membrane receptor.

Summary points

- Epidemiological studies suggest that low serum 25(OH)D is associated with reduced insulin sensitivity.
- Results from clinical trials and animal studies support that vitamin D intervention may improve insulin resistance.
- Vitamin D may influence insulin sensitivity through its ability to enhance muscle mass and function.
- Expression of the 25(OH)D 1α-hydroxylase enzyme in extra-renal tissues relevant to insulin sensitivity such as muscle, adipose tissue and pancreas may allow for local conversion of 25(OH)D to 1α,25$(OH)_2$D, leading to autocrine/paracrine responses.
- There is evidence to suggest that 1α,25-dihydroxyvitamin D (1α,25$(OH)_2$D) regulates insulin sensitivity at key points in the insulin signaling pathway including increased protein expression of the insulin receptor and tethering and fusion of the GLUT4 glucose transporters to the cell membrane, thereby enhancing insulin signaling and facilitating glucose uptake.
- Parathyroid hormone (PTH) and 1α,25$(OH)_2$D are major regulators of serum calcium. Elevated serum parathyroid hormone is associated with impaired insulin sensitivity, and high serum 25(OH)D may enhance insulin sensitivity through its ability to lower serum PTH.
- Increased adiponectin and osteocalcin are associated with improved insulin sensitivity and secretion, and studies suggest a positive relationship between these and serum 25(OH)D.
- Vitamin D is essential for insulin synthesis and secretion in pancreatic β-cells.

26. Vitamin D and insulin sensitivity

B. Larrick, S. Donkin and D. Teegarden
Interdepartmental Nutrition Program, Purdue University, West Lafayette, IN 47907, USA;
teegarden@purdue.edu

Abstract

Insulin resistance is characterized by an insufficient response of the body to insulin secretion, and is associated with a cluster of metabolic disorders that are collectively referred to as the insulin resistance syndrome. Features associated with insulin resistance include glucose intolerance, hyperinsulinema, hyperglycemia, hypertriglyceridemia, and hypertension, among others, and together these increase the likelihood of developing type 2 diabetes. Evidence from epidemiological, clinical, and tissue- and cell-based studies suggest a role of vitamin D in the prevention of insulin resistance. Serum levels of 25-hydroxyvitamin D, an indicator of vitamin D status, have been associated with improved glucose homeostasis, and data from clinical intervention studies support that treatment with 1α,25-dihydroxyvitamin D, the hormonally active form of vitamin D, may improve insulin sensitivity. Investigations of the mechanisms by which vitamin D improves insulin sensitivity suggest that vitamin D may have direct effects on insulin synthesis and secretion, as well as insulin-sensitizing effects on target tissues. Defining the mechanisms by which vitamin D may improve insulin sensitivity and glucose homeostasis is critical to understanding how vitamin D may be effective in preventing the development of insulin resistance, and its progression to type 2 diabetes.

Keywords: vitamin D, 1α,25$(OH)_2$D, insulin resistance, homeostatic model assessment

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_26, © Wageningen Academic Publishers 2013

Abbreviations

$1\alpha,25(OH)_2D$	1α,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AMPK	AMP-activated protein kinase
BMI	Body mass index
CI	Confidence interval
FFA	Free fatty acid
GLUT4	Glucose transporter type 4
HbA_{1c}	Hemoglobin A_{1c}
HOMA-IR	Homeostatic model assessment of insulin resistance
IFG	Impaired fasting glucose
IRS-1	Insulin receptor substrate-1
IRS-2	Insulin receptor substrate-2
OGTT	Oral glucose tolerance test
PHPT	Primary hyperparathyroidism
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
PKC	Protein kinase C
PTH	Parathyroid hormone
SH2 domain	Src homology 2 domain
VDR	Vitamin D receptor

26.1 Vitamin D metabolism

Vitamin D3 (cholecalciferol) is a biologically inert prohormone made endogenously from 7-dehydrocholesterol in the skin upon exposure to ultraviolet light. To become an active hormone, vitamin D3 is metabolized first in the liver to its major circulating form 25(OH)D, which circulates bound to serum vitamin D binding protein. In the kidney 25(OH)D is converted to its hormonal form, $1\alpha,25(OH)_2D$, which functions through the nuclear VDR (DeLuca, 2004). Formation of 25(OH)D by hepatic vitamin D-25-hydroxylase (CYP27) appears to be only loosely regulated, and is used as an indicator of vitamin D status. However, conversion of 25(OH)D to $1\alpha,25(OH)_2D$ by renal 1α-hydroxylase (CYP1α) is under tight regulation, influenced by serum PTH, $1\alpha,25(OH)_2D$, and calcium levels (Jones *et al.*, *19*98). Degradation of $1\alpha,25(OH)_2D$ is induced via a negative-feedback mechanism, initiated by 24-hydroxylation of $1\alpha,25(OH)_2D$ by 24-hydroxylase (CYP24). 24-Hydroxylation results in the metabolism of $1\alpha,25(OH)_2D$ to calcitroic acid, which is eliminated primarily through the bile (DeLuca, 2008) (Figure 26.1).

Though the activity of renal 1α-hydroxylase is tightly controlled by PTH, 1α-hydroxylase enzymes have been identified in several extra-renal tissues, including skin, colon, lung, bone, prostate, intestine, pancreatic islets, vasculature, liver, brain, muscle and adipose tissue, among others, which may allow for local production of $1\alpha,25(OH)_2D$ (Hewison *et al.*, 2007; Li *et al.*, 2008;

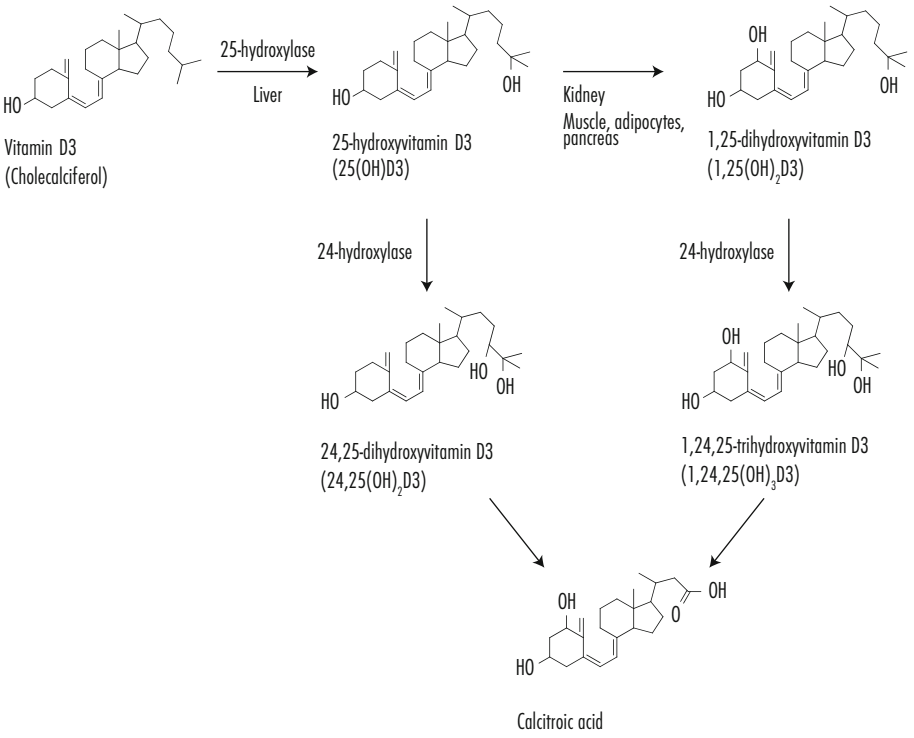

Figure 26.1. The metabolism of vitamin D.

Zehnder *et al.*, 2001). Additionally, these extra-renal 1α-hydroxylase enzymes do not appear to be regulated by PTH (Flanagan *et al.*, 2003; Young *et al.*, 2004), suggesting that elevated 25(OH)D status may lead to local production of 1α,25$(OH)_2$D independent of calcium status, potentially yielding autocrine/paracrine responses such as acute improvements in insulin resistance.

1α,25$(OH)_2$D induces genomic responses through interaction with the nuclear VDR, as well as non-transcriptional responses involving activation of transmembrane signal transduction pathways via a putative novel membrane receptor, including activation of the adenylyl cyclase-cAMP-protein kinase A and phospholipase C-diacylglycerol (DAG)-inositol trisphosphate-PKC signal transduction pathways and the Ca^{2+} messenger system (Boland, 2011). 1α,25$(OH)_2$D is involved in biological processes such as cell proliferation, differentiation, apoptosis, and muscle contraction (Boland *et al.*, 2005), and an emerging body of evidence also suggests the involvement of 1α,25$(OH)_2$D in the improvement of insulin sensitivity, which will be the focus of this chapter.

26.2 Insulin resistance

Insulin resistance is characterized by a subnormal response of the body to insulin, and is an early predictor of type 2 diabetes. Under normal conditions, circulating levels of blood glucose

are under tight homeostatic regulation. When blood glucose levels rise in response to dietary glucose, insulin secretion is stimulated in pancreatic β-cells to promote peripheral glucose uptake. Conversely, when concentrations of blood glucose are too low, glucagon secretion from pancreatic α-cells is stimulated to maintain normoglycemia (Erkelens, 2001; Muniyappa *et al.*, 2008).

Initiation of the insulin signaling cascade begins when insulin binds to the insulin receptor (Taniguchi *et al.*, 2006). The insulin receptor belongs to the family of receptor tyrosine kinases, which, in addition to the insulin receptor, includes insulin-like growth factor-1, vascular endothelial growth factor, fibroblast growth factor, and epidermal growth factor (Fantl *et al.*, 1993; Ullrich and Schlessinger, 1990). The insulin receptor consists of two extracellular α-subunits, each of which are linked to one of two intracellular β-subunits having tyrosine kinase domains. In insulin-responsive tissues, binding of insulin to the α-subunit of the insulin receptor induces allosteric interactions between the two receptor dimers. This allows for increased tyrosine kinase activity of the insulin receptor β-subunits, causing autophosphorylation of tyrosine residues in the insulin receptor, and subsequent phosphorylation of IRS-1 and IRS-2 (Taniguchi *et al.*, 2006).

Both IRS-1 and IRS-2 have phosphotyrosine-binding and pleckstrin-homology domains, and serve as docking platforms for other signaling proteins that have SH2 domains (Taniguchi *et al.*, 2006). These phosphotyrosine- and pleckstrin-homology binding domains have an affinity for phosphatidylinositol lipids and proteins such as PKC, and are highly involved in cell signal transduction pathways (White, 2002). At least twenty tyrosine residues have been identified as insulin-stimulated phosphorylation sites, including Tyr-460, -608, -628, -895, -939, -987, -1172, and -1222 (numbers correspond to rat IRS-1 sequence) (Sun *et al.*, 1993). Following phosphorylation of several tyrosine residues within the IRS proteins the intracellular signaling molecule PI3K, which has a SH2 domain and can associate with IRS-1, is activated. This results in the subsequent generation of the second messenger phosphatidylinositol-3,4,5-trisphosphate which binds phosphoinositide-dependent protein kinase 1, resulting in the downstream activation of PKB/Akt and PKC (Bomfeldt and Tabas, 2011; Henriksen *et al.*, 2011; Schwanstecher and Schwanstecher, 2011; Taniguchi *et al.*, 2006; White, 2002).

This signaling event results in glucose uptake via GLUT4 glucose transporters in both skeletal muscle and adipose tissue, however the effects of this insulin-stimulated signaling event differ between muscle and adipose tissue. In adipocytes, in a non-insulin-stimulated state intracellular GLUT4 vesicles travel along a microtubule network covering the entire plasma membrane, to which the vesicles periodically tether in a loose, temporary fashion. In response to insulin, GLUT4 vesicles tether tightly and fuse to the plasma membrane, increasing the concentration of membrane GLUT4 transporters and allowing for glucose uptake (Lizunov *et al.*, 2005). In muscle, the major site of glucose disposal, insulin stimulates local fusion of GLUT4 vesicles that are already 'pre-tethered' to the sarcolemma, or muscle cell membrane, allowing for glucose uptake, rather than stimulating the recruitment of intracellular GLUT4 vesicles (Lizunov *et al.*, 2012).

Systemic actions of insulin include inhibition of hepatic gluconeogenesis and glycogenolysis, inhibition of FFA release from adipose tissue, and stimulation of fatty acid, protein, and triglyceride biosynthesis (Brown and Goldstein, 2008; Muniyappa *et al.*, 2008; Reaven, 2005a; Schwanstecher and Schwanstecher, 2011) and are mediated through the signaling molecules described above. Insulin binding to its receptor also activates other signaling pathways including mitogen-activated protein kinase, which is involved in mitogenesis, survival, and cell differentiation (Avruch, 1998; Taniguchi *et al.*, 2006). There are several points in the insulin signaling cascade at which disruption in signal transduction may occur in insulin resistance, such as negative regulation of the insulin receptor, of the IRS proteins, and of PKB/AKT kinases. Negative regulators of the insulin receptor include receptor serine phosphorylation and protein tyrosine phosphatases, which dephosphorylate critical tyrosine residues thereby reducing the receptor's activity. Additionally, suppressor of cytokine signaling proteins sterically hinder the interaction between the insulin receptor and IRS proteins, and ligand-induced internalization and degradation of the insulin receptor both contribute to impaired insulin sensitivity. Sustained inhibition of IRS proteins may occur via ubiquitin-targeted proteosome-mediated degradation (White, 2002), and IRS proteins may undergo serine phosphorylation at Ser-24, -267, -302, -307, -318, -332, -357, -408, -522, -612, -632, -662, -731, -789, and -1099/1100 (Weigert *et al.*, 2008) in response to insulin, free fatty acids, and cytokines resulting in short-term inhibition of insulin-induced cell signaling (White, 2002). Hyperinsulinemic states may also interfere with insulin signaling by reducing expression of IRS-1 (Schwanstecher and Schwanstecher, 2011; Taniguchi *et al.*, 2006).

Disruptions in insulin receptor signaling, or chronic activation of insulin receptor pathways in response to elevated FFA, glucose, insulin, cytokines, or other growth factors, act to reduce sensitivity to insulin and impairs both insulin-mediated glucose disposal and control of hepatic glucose output (Bomfeldt and Tabas, 2011; Muniyappa *et al.*, 2008; Prentki and Nolan, 2006). Pancreatic β-cells can compensate for a subnormal response to insulin by increasing insulin secretion to maintain normoglycemia, but this compensatory hyperinsulinemia, combined with resistance to insulin signaling, increases one's risk of developing hypertension and dyslipidemia (Prentki and Nolan, 2006; Reaven, 2005b). The extent to which pancreatic β-cells are capable of maintaining hypersecretion of insulin varies between individuals, and it is in individuals with 'susceptible' β-cells that loss of β-cell function and progressive loss of β-cell mass due to apoptosis occurs. This leads to poor glucose tolerance, measured as the time required to dispose of an oral glucose load, and the eventual development of type 2 diabetes (Henriksen *et al.*, 2011; Prentki and Nolan, 2006; Schwanstecher and Schwanstecher, 2011). Several gene variants associated with insulin sensitivity and impaired β-cell function have been identified, and include the P12A polymorphism in peroxisome proliferator-activated receptor γ, and mutations in β-cell transcription factors and metabolic regulatory proteins such as hepatocyte nuclear factor-4α, glucokinase, and pancreatic and duodenal homeobox-1. It is thought that enhanced susceptibility to environmental factors such as overnutrition, obesity, and stress due to a cluster of genetic variations may contribute to the onset of β-cell failure and development of type 2 diabetes (Kahn *et al.*, 2006; Muoio and Newgard, 2008).

Hyperinsulinemia and impaired sensitivity to insulin precede the development of type 2 diabetes, and the cluster of symptoms that are found to be associated with hyperinsulinema and insulin resistance have been collectively termed the insulin resistance syndrome. Features associated with insulin resistance include glucose intolerance, hyperglycemia, elevated triacylglycerol concentrations, reduced high-density lipoprotein concentrations, hypertension, increased procoagulant factors and markers of inflammation, and impaired endothelial function (Frayn, 2010; Muniyappa *et al.*, 2008; Reaven, 2005b). These conditions associated with insulin resistance increase the risks for developing type 2 diabetes, cardiovascular disease, hypertension, and cancer (Reaven, 2005b). Several studies also indicate a significant association of hyperinsulinemia with elevated risk of coronary artery disease (Haffner, 1999).

26.3 Epidemiological evidence for a role of vitamin D in insulin resistance

26.3.1 Glucose homeostasis, insulin sensitivity and 25(OH)D levels

Measures of glucose homeostasis in both children (Buyukinan *et al.*, 2012) and adults (Alvaraz and Ashraf, 2010; Chagas *et al.*, 2012; Mitri *et al.*, 2011; Palomer *et al.*, 2008; Pittas *et al.*, 2007a) suggest an inverse relationship between serum 25(OH)D, the preferred indicator of vitamin D status and insulin sensitivity. In a cross-sectional analysis of the Third National Health and Nutrition Examination Survey (n=6,228), serum 25(OH)D was negatively correlated with fasting glucose levels in non-Hispanic whites (OR 0.25) and Mexican blacks (OR 0.17) after adjusting for age, sex, BMI, leisure activity, and time of the year. Additionally, serum 25(OH)D was inversely correlated with fasting and 2-hour glucose, fasting insulin and HOMA-IR (a measure of insulin resistance and β-cell function) in Mexican Americans, with a trend towards significance in non-Hispanic whites (P=0.06). There were no observed associations between serum 25(OH)D and measures of glucose homeostasis in non-Hispanic blacks, however, suggesting a potential race-specific relationship between serum 25(OH)D and glucose levels (Scragg *et al.*, 2004). Additional analyses of the Third National Health and Nutrition Examination Survey data found inverse associations of serum 25(OH)D status with several components of the metabolic syndrome including abdominal obesity, hypertriglyceridemia, and hyperglycemia (Ford *et al.*, 2005) as well as prediabetes (defined as 2-hour glucose concentration of 140-199 mg/dl, fasting glucose concentration of 110-125 mg/dl, or HbA_{1c} value of 5.7-6.4%) after age, sex, ethnicity, and other multivariable adjustments (Shankar *et al.*, 2011). Serum 25(OH)D levels were found also to be inversely related to fasting serum glucose in postmenopausal women (n=753), after controlling for BMI, weight, and age (Need *et al.*, 2005).

Several additional epidemiological studies indicate an association between serum 25(OH)D and glucose homeostasis. A 5-year follow-up examination of men and women (n=4,296, aged 30-65 years) that had participated in the Inter99 study found that individuals with low serum 25(OH)D levels had increased 2-hour glucose and insulin, and increased degree of insulin resistance than those with higher 25(OH)D levels (Husemoen *et al.*, 2012). A cross-sectional analysis of participants of the Kuopio Ischaemic Heart Disease Risk Factor Study (n=1,756;

aged 53-73) found age, gender, and examination year-adjusted serum 25(OH)D to be inversely associated with fasting serum insulin, fasting serum glucose, and OGTT 2-hour glucose levels in both men and women. After further multivariable adjustments for age, gender, examination period, BMI, waist-to-hip ratio, smoking, activity, food intake, and diabetes family history, fasting serum insulin and glucose associations were attenuated, but 2-hour OGTT remained significant, suggesting that low serum 25(OH)D may be associated with impaired glucose and insulin metabolism (Hurskainen *et al.*, 2012). Low serum vitamin D was found to be associated with insulin insensitivity in some, but not all, studies (Pittas *et al.*, 2007a). Inconsistencies in the literature are likely due to differences in subject populations, forms of vitamin D administered, and length of interventions.

26.3.2 Dairy intake and insulin secretion and glucose homeostasis

Intake of fortified dairy, the primary dietary source of vitamin D, may also affect insulin secretion and glucose homeostasis. In a prospective analysis of the Health Professionals Follow-up Study (males, n=41,254), after 12 years of follow-up, the relative risk for type 2 diabetes in men in the top quintile of dairy intake was 0.77 (95% CI 0.62, 0.95) compared to those in the lowest quintile, corresponding to a 9% reduction in risk for type 2 diabetes with each serving per day increase in dairy (Choi *et al.*, 2005). Subsequent cross-sectional analysis of male participants in the Health Professionals Follow-up Study and female participants in the Nurses' Health Study examined the relationship between plasma 25(OH)D concentrations and calcium intake and fasting concentrations of C-peptide, a marker of endogenous insulin secretion. Plasma concentrations of C-peptide for the highest tertiles of calcium intake and serum 25(OH)D concentrations compared to the lowest tertiles were 35% lower in men (P=0.03) and 12% lower in women (P=0.01), suggesting that high calcium intake and serum 25(OH)D status are associated with decreased insulin secretion. Nonsignificant negative trends were observed between calcium intake and C-protein concentrations in women (P=0.05), and between serum 25(OH)D and C-protein concentrations in men (P=0.08) (Wu *et al.*, 2009). Loss of pancreatic β-cell function leads to poor glucose tolerance and the eventual development of type 2 diabetes, and there is a large body of evidence to suggest a role of $1\alpha,25(OH)_2D$ in β-cell secretory function (Pittas *et al.*, 2007a), though this relationship was not observed in all studies (Husemoen *et al.*, 2012). Taken together, the epidemiological studies predominantly suggest a relationship between serum 25(OH)D status and insulin action to control glucose homeostasis.

26.3.3 Relationship between vitamin D and insulin resistance in children

Investigations of potential relationships between vitamin D and insulin resistance in children are of particular interest, as the prevalence of type 2 diabetes among children and adolescents has increased significantly over the past two decades (Kaufman and Shaw, 2007). As in adult populations, vitamin D insufficiency and deficiency are common among youth, and a significant relationship between serum 25(OH)D levels and obesity in children, likely due to the sequestering of vitamin D in fat stores, has been found in some (Alemzadeh *et al.*, 2008; Olson *et al.*, 2012) but not all (Rajakumar *et al.*, 2008; Roth *et al.*, 2011) studies. Obese children may be more likely to

be sedentary with reduced sunlight exposure from outdoor physical activity (Roth *et al.*, 2011), and are likely to have significantly lower dietary vitamin D intakes than nonobese children (Rajakumar *et al.*, 2008), contributing to the risk of both vitamin D deficiency and obesity-associated metabolic abnormalities.

In children there is strong evidence to support an inverse relationship between vitamin D status and insulin resistance. An assessment of obese and nonobese children (n=156, age 11.9±2.7 yr, 51% female) in Germany found that 76% of subjects were 25(OH)D-deficient (<20 ng/ml, or <50 nM), and higher insulin, HOMA-IR, and HbA_{1c} were found in obese children with lower serum 25(OH)D concentrations after adjusting for gender, age, and BMI (Roth *et al.*, 2011). Further, cross-sectional results from obese (n=411, age 11.7±2.6 yr, 57% female) and nonobese (n=87, age 11.2±3.0 yr, 68% female) children show that those who were obese were more likely to have lower serum 25(OH)D levels ($P<0.001$), and that 25(OH)D concentrations were negatively correlated with HOMA-IR (r=-0.19; $P<0.001$) and 2-hr glucose (r= -0.12; $P=0.04$) after adjusting for BMI and age (Olson *et al.*, 2012). There was no correlation between serum 25(OH)D levels and HbA_{1c} in this study however a significant inverse relationship was reported for serum 25(OH)D levels and HbA_{1c} by others (Roth *et al.*, 2011). Finally, a multivariate linear regression analysis of data from nonobese Korean children (n=188, age = 12-13 yr) showed that serum 25(OH)D concentrations were inversely associated with HOMA-IR ($P=0.025$), as well as serum triglycerides ($P=0.037$) and LDL cholesterol ($P=0.045$) (Shin *et al.*, 2012). Taken together, these data suggest a relationship between serum 25(OH)D and measures of insulin resistance in a pediatric populations. Further studies to support this, as well as investigations of vitamin D deficiency and insufficiency on measures of insulin resistance in adulthood, are warranted.

26.4 Results of clinical interventions

Several intervention studies support an effect of vitamin D supplementation on glucose homeostasis and insulin resistance. In a double-blind, randomized, controlled trial designed for bone-related outcomes, Caucasian, nondiabetic adults (n=314) received supplementation of 500 mg calcium citrate plus 700 IU vitamin D3 daily for three years. Post hoc analysis revealed that after three years of calcium and vitamin D3 supplementation subjects with IFG at baseline had a lower increase in fasting plasma glucose and HOMA-IR compared with those with IFG at baseline given the placebo. For individuals with normal fasting glucose at the time of baseline determination, vitamin D3 and calcium supplementation had no significant effect on fasting plasma glucose or HOMA-IR, suggesting that for individuals with IFG, treatment with vitamin D3 may be effective in attenuating increases in glycemia and insulin resistance that occur over time (Pittas *et al.*, 2007b). Similarly, supplementation of 4,000 IU vitamin D3 daily for 6 months significantly improved insulin sensitivity and insulin resistance in vitamin D deficient, insulin resistant South Asian women (n=81) (Von Hurst, *et al.*, 2010). In another study, the effect of intravenous $1\alpha,25(OH)_2D3$ therapy on insulin and lipid metabolism was examined in uremic patients on maintenance hemodialysis (n=16) as compared to no infusion controls (n=7). Four weeks of intravenous $1\alpha,25(OH)_2D3$ therapy (1.8±0.3 μg) corrected glucose intolerance, insulin

resistance, and hypoinsulinemia, as well as hypertriglyceridemia in these patients (Mak, 1998). The effects of a single, large-dose intramuscular injection of vitamin D3 (300,000 IU) on glucose tolerance and insulin resistance in women with gestational diabetes (n=45) were observed in a randomized controlled trial. An intramuscular injection of vitamin D3 was administered 3-10 days postpartum, and after a 3-month follow-up period, treatment with vitamin D3 was found to prevent the increase in HOMA-IR, and decrease in quantitative insulin sensitivity check index, that were observed in the control group (Mozaffari-Khosravi *et al.*, 2012). This suggests that large, single-dose treatment with vitamin D3 may also be effective in improving indices of insulin resistance in mothers with gestational diabetes after delivery.

However, not all intervention trials report an effect of vitamin D treatment on insulin response (George *et al.*, 2012). In a double-blind, placebo-controlled, cross-over trial of 1α,25$(OH)_2$D3 treatment (1 μg/day for 4 days) of diabetic subjects (n=35), no effect of 1α,25$(OH)_2$D3 was observed on fasting or stimulated glucose, insulin, C-peptide, or glucagon concentrations (Orwoll *et al.*, 1994). Similarly, in another randomized, placebo-controlled, double-blind trial with women with polycystic ovary syndrome (n=50), three oral doses of vitamin D3 (50,000 IU) administered at 20 day intervals did not significantly affect fasting serum insulin or glucose levels, or HOMA-IR (Ardabili *et al.*, 2012). This trial is confounded by a relatively short duration and low dose of treatment therefore additional measures may be necessary to determine the role of vitamin D in treating insulin resistance associated with polycystic ovary syndrome.

The largest intervention study to examine the effects of dietary vitamin D and calcium on health outcomes is the Women's Health Initiative, and risk of diabetes was examined in secondary analysis of this intervention. In this study, postmenopausal women without diabetes at baseline (n=33,951) were given either a placebo or 1000 mg calcium plus 400 IU vitamin D3, for a median follow-up time of 7 years. In this study, calcium plus vitamin D supplementation did not affect diabetes risk. The hazard ratio for incident diabetes associated with the calcium/vitamin D treatment group was 1.01 (95% CI 0.94-1.10) (De Boer *et al.*, 2008). The data infer a lack of vitamin D effect however it should be noted that the effects of vitamin D were evaluated at a single dose and frequency of vitamin D administered. Evidence reviewed here suggests that both dose and frequency of vitamin D supplementation may determine efficacy in normalizing glucose homeostasis.

26.5 Association of vitamin D with muscle mass and function

Skeletal muscle is the major site of insulin-dependent glucose uptake from the blood. Therefore, muscle mass is an important determinant of insulin sensitivity, and skeletal muscle insulin resistance is critical in the development of type 2 diabetes. Vitamin D clearly plays a role in muscle function, as evidenced by classic symptoms of vitamin D deficiency including decreased muscle strength, changes in gait, inability to ascend stairs, difficulties in rising from a chair and diffuse muscle pain (Pfeifer *et al.*, 2002). Data from epidemiological, animal and cell culture studies suggest a role for vitamin D in enhancing insulin sensitivity and insulin-induced signal

transduction in skeletal muscle. Meta-analyses of randomized clinical trials conclude that vitamin D supplementation significantly improves body sway and fall incidence rate, as well as measures of balance, strength, and gait in elderly populations (Bischoff-Ferrari *et al.*, 2004; Muir and Montero-Odasso, 2011). Diets high in vitamin D have been associated with greater muscle mass in rodents (Siddiqui *et al.*, 2008), and treatment of C2C12 skeletal muscle cells with 1α,25$(OH)_2$D enhanced myogenic differentiation at the cellular level (Garcia *et al.*, 2011). In C2C12 skeletal muscle cells at the proliferative and early differentiated stages, 1α,25$(OH)_2$D activates Akt by phosphorylation of Ser473 in a time-dependent manner, from 5-60 minutes (P<0.05) in proliferative cells, and at 48 and 60 hours in C2C12 cells in early stages of differentiation (Buitrago *et al.*, 2012). This up-regulation is likely to be at least in part responsible for the enhanced myogenic differentiation observed in skeletal muscle cells treated with 1α,25$(OH)_2$D, and together, these results suggest that vitamin D may promote insulin sensitivity through its ability to enhance insulin signaling in, and improve mass of, skeletal muscle.

Exposure of skeletal muscle cells to free fatty acids such as palmitate will induce insulin resistance through increased serine/threonine phosphorylation of IRS-1. This results in a reduced capacity of IRS-1 to activate PI3K and Akt, causing impaired insulin signaling event. It has been shown that elevated levels of FFA in circulation play a key role in the pathogenesis of type 2 diabetes (Boden, 1997), and exposing cultured skeletal muscle cells and isolated skeletal muscle tissue to FFA such as palmitate will induce insulin resistance (Dimopoulos *et al.*, 2006; Hirabara *et al.*, 2007; Jové *et al.*, 2006; Schmitz-Peiffer *et al.*, 1999). Treatment of C2C12 myotubes with 1 mM palmitate produces an increase in serine phosphorylation of IRS-1 associated with blunted insulin signal transduction, and a decrease in tyrosine phosphorylation of IRS-1, which is necessary to propagate the insulin signaling cascade. In one study, co-treatment of C2C12 skeletal muscle cells with 10 nM 1α,25$(OH)_2$D and 1 mM palmitate prevented these changes in IRS-1 phosphorylation states, as well as the decrease in Akt activation that is observed in FFA-treated, insulin-resistance cells. Co-treatment of the C2C12 cells with 1α,25$(OH)_2$D and palmitate resulted in increased insulin-stimulated glucose uptake, and inhibited FFA-induced c-Jun N-terminal kinase phosphorylation, which is thought to be implicated in the pathogenesis of insulin resistance (Zhou *et al.*, 2008). Inconsistencies in results from clinical trials warrants additional research to establish a clinically relevant relationship between serum 25(OH)D and muscle mass in humans (Marantes *et al.*, 2011), but available data suggest that the insulin-sensitizing effects of vitamin D may be due at least in part to gains in insulin-responsive muscle tissue mass and improved insulin signaling.

26.6 Molecular mechanisms underlying vitamin D action on insulin sensitivity

There is evidence from animal, tissue, and cell model studies to support an effect of vitamin D on insulin action at several levels, including direct effects at target tissues to alter sensitivity to insulin, as well as through local conversion of 25(OH)D to 1α,25$(OH)_2$D within the pancreatic β-cell to alter insulin release (Pittas *et al.*, 2007a). Presence of the VDR (Johnson *et al.*, 1994) and expression of 1α-hydroxylase (Bland *et al.*, 2004) in pancreatic β-cells, and the identification of the vitamin D response element in the human insulin gene promoter (Maestro *et al.*, 2003)

suggest a direct effect of vitamin D on insulin secretion. Further, mice lacking a functional VDR have impaired insulin secretion (Zeitz *et al.*, 2003), highlighting the involvement of vitamin D in insulin signaling. Several studies have shown that vitamin D-deficient rats present with impaired glucose tolerance and reduced insulin secretion, and repletion of vitamin D restores impaired glucose tolerance and insulin release (Cade and Norman, 1986; Kadowaki and Norman, 1984). Further, ob/ob mice, which exhibit homozygous mutations in the gene for leptin resulting in obesity, hyperphagia, hyperglycemia and hyperinsulinemia, treated with $1\alpha,25(OH)_2D$ improved hyperinsulinemia and hyperglycemia (Kawashima and Castro, 1981). These results support a role of vitamin D in maintaining normoglycemia, and it is hypothesized that the impact of $1\alpha,25(OH)_2D$ on insulin release may occur through increased conversion of pro-insulin to insulin (Bourlon *et al.*, 1996, 1999), or by increasing pancreatic β-cell replication and resistance to apoptosis.

Additionally, $1\alpha,25(OH)_2D$ may affect insulin release by normalizing intracellular Ca^{2+} levels (Takiishi *et al.*, 2012), the elevation of which in human adipocytes, leukocytes, and platelets is associated with insulin resistance (Draznin, 1993; Resnick, 1992). In rodents, measures which increase intracellular calcium in muscle and adipocytes are associated with impaired insulin signaling, likely through an impaired ability of insulin to activate phosphoserine phosphatase-1 (Begum *et al.*, 1992). Parathyroid hormone, synthesized in the chief cells of the parathyroid, acts to increase serum calcium levels, and its synthesis fluctuates inversely in response to serum 25(OH)D and calcium levels (D'Amour, 2012). Parathyroid hormone can act to increase intracellular calcium levels in adipocytes and muscle, and secondary hyperparathyroidism resulting from low calcium intake and vitamin D deficiency may therefore contribute to insulin resistance (Draznin, 1993; McCarty, 2006). Elevated intracellular calcium levels may contribute to insulin resistance by increasing binding of calmodulin to IRS-1, thus interfering with insulin-stimulated tyrosine phosphorylation of IRS-1 and the subsequent activation of PI3K (Li *et al.*, 2000). Vitamin D may therefore exert its insulin-sensitizing effects through its ability to regulate intracellular calcium levels by reducing serum levels of PTH.

26.6.1 Vitamin D increases protein expression of the insulin receptor

Another mechanism by which vitamin D may elicit improvements in insulin sensitivity is through increased abundance of the insulin receptor protein. A vitamin D response element has been identified in the promoter region of the human insulin receptor gene (Maestro *et al.*, 2003), and increases in IR mRNA expression have been observed upon treatment with vitamin D in both cell (Maestro *et al.*, 2000) and rodent (Siddiqui *et al.*, 2008) models. Later work by Maestro *et al.* (2002) demonstrated that $1\alpha,25(OH)_2D$ induces the transcriptional activation of the human IR gene in U-937 promonocytic cells through the PI3K signaling system, and that $1\alpha,25(OH)_2D$-mediated increases in IR expression potentiates the action of insulin on glucose oxidation, providing a possible mechanism by which $1\alpha,25(OH)_2D$ may affect insulin sensitivity.

26.7 Additional regulators of insulin sensitivity

26.7.1 Parathyroid hormone

Parathyroid hormone and the vitamin D metabolite 1α,25$(OH)_2$D are major hormonal regulators of calcium homeostasis. Low serum calcium is sensed by Ca-sensing receptors of the parathyroid gland, which signal for a release of PTH into circulation (Hebert *et al.*, 1997). PTH stimulates the renal enzyme 1α-hydroxylase to convert 25(OH)D to its hormonally active form, 1α,25$(OH)_2$D, which acts on bone, kidney, and intestine to regulate calcium homeostasis (Zierold *et al.*, 2003). Consequently, a dietary calcium load results in an acute lowering of serum PTH, while higher vitamin D and calcium status are associated with lower serum levels of PTH (Harkness and Cromer, 2005; Pepe *et al.*, 2005).

In addition to regulation of calcium homeostasis, evidence suggests that PTH may negatively influence glucose homeostasis and insulin sensitivity, in part by influencing body fat mass. In a meta-analysis of seventeen studies investigating the relationship between individuals with primary hyperparathyroidism and adiposity, subjects with primary hyperparathyroidism were 3.34 (95% CI 1.97, 4.17, P<0.00001) kg heavier compared with controls, or had an increased BMI of 1.13 (95% CI -0.29 to 2.55; P=0.12) kg/m^2 compared with controls (Bolland *et al.*, 2005). Additionally, studies of young adults between the ages of 20 and 35, as well as those aged 65 and older without PHPT also show associations between PTH and BMI and body fat percentage (Bell *et al.*, 1985; Snijder *et al.*, 2005), suggesting that serum PTH may be involved in regulating adiposity, a major risk factor for diabetes and insulin insensitivity.

Parathyroid hormone may also directly influence glucose homeostasis. Serum PTH was found to be inversely associated with hyperglycemia in men (Reis *et al.*, 2007), and with insulin sensitivity index in normotensive adults (Chiu *et al.*, 2000). Further, individuals with PHPT were found to have impaired glucose intolerance and insulin sensitivity compared to individuals without PHPT (Kumar *et al.*, 1994). This negative effect of PTH on insulin sensitivity is hypothesized to occur through a variety of mechanisms, including suppressed pancreatic insulin release (Perna *et al.*, 1990), increased intracellular calcium levels leading to reduced insulin-stimulated glucose uptake in adipocytes (Ni *et al.*, 1994; Reusch *et al.*, 1991), and by inducing insulin resistance in adipocytes (Chang *et al.*, 2009). PTH may negatively affect insulin sensitivity through increasing adiposity and interfering with insulin signaling, and vitamin D may improve insulin sensitivity through its ability to lower serum PTH levels.

26.7.2 Adiponectin

Though muscle tissue is the body's main source of glucose disposal, adipose tissue also plays a central role in regulating insulin secretion, insulin action, and glucose and lipid metabolism. Secretory products of adipose tissue include enzymes, growth factors, cytokines, and several hormones involved in fatty acid and glucose metabolism (Mora and Pessin, 2002). Adiponectin, an adipose-derived peptide hormone, is associated with improved glucose tolerance, and reduced

risk of obesity, cardiovascular disease, insulin resistance and type 2 diabetes (Kishida *et al.*, 2012; Tishinsky *et al.*, 2012). Serum adiponectin and mRNA expression in adipose tissue are decreased in obese and type 2 diabetic humans (Arita *et al.*, *19*99, Weyer *et al.*, 2001), and in obese rodent models, administration of adiponectin improves insulin sensitivity and glucose homeostasis (Dzamko and Steinberg, 2009; Yamauchi *et al.*, 2001). Adiponectin also serves as an activator of AMPK, resulting in increased fatty acid β-oxidation, mitochondrial biogenesis, and insulin sensitivity in skeletal muscle, as well as decreased hepatic gluconeogenesis (Dzamko and Steinberg, 2009). Several studies report significant correlation between serum 25(OH)D status and adiponectin levels in both children (Roth *et al.*, 2011) and adults (Gannagé-Yared *et al.*, 2009; Nimitphong *et al.*, 2009; Vaidya *et al.*, 2011). Additionally, treatment of adults with type 2 diabetes (n=90) with 1000 IU vitamin D3 or 1000 IU vitamin D3 plus 500 mg calcium per day for 12 weeks produced a significant increase in serum adiponectin levels (Neyestani *et al.*, 2012). Adiponectin may therefore be implicated in the observed effects of vitamin D on insulin resistance.

26.7.3 AMP-activated protein kinase

AMP-activated protein kinase is a ubiquitous serine/threonine protein kinase that acts as a sensor of cellular energy status. It is activated by an increased AMP:ATP ratio (low cellular energy state), and acts to inhibit anabolic, ATP-consuming cellular processes while stimulating catabolic, ATP-generating processes (Towler and Hardie, 2007; Viollet *et al.*, 2003). AMPK may increase insulin sensitivity by altering metabolism to promote fat oxidation and reduce muscle triglyceride storage, thereby reducing risk factors associated with insulin resistance. These risk factors may be reduced through increasing mitochondrial biogenesis, which is of relevance as individuals who are at risk of developing type 2 diabetes appear to have reduced mitochondrial function (Hardie, 2011; Lowell and Shulman, 2005). AMPK activation in skeletal muscle may also contribute to improved insulin signaling. Primary cardiac and skeletal myocyte cultures subject to insulin- and glucose-free conditions exhibited AMPK-dependent, insulin-independent activation of the insulin receptor, providing a direct link between AMPK and the insulin signaling pathway (Chopra *et al.*, 2012). The rise in AMP:ATP ratio that accompanies muscle contraction also triggers AMPK activation (Winder and Hardie, 1996), which in muscle results in increased GLUT4 expression and increased glucose uptake (Zheng *et al.*, 2001). This increased GLUT4 expression occurs even in insulin-resistant individuals, allowing for greater insulin-induced glucose uptake even without changes in insulin sensitivity (Hardie, 2011). Though no direct link between vitamin D and AMPK has been tested, Vitamin D is implicated in muscle mass, strength, and function (Dirks-Naylor and Lennon-Edwards, 2011; Teegarden and Donkin, 2009), and may therefore contribute to whole-body insulin sensitivity through enhanced contraction-stimulated, AMPK-dependent glucose uptake by skeletal muscle.

26.7.4 Osteocalcin

In addition to skeletal muscle and adipose tissue, the skeleton also exerts endocrine regulation of whole-body glucose metabolism. In its uncarboxylated state, osteocalcin, a hormone secreted exclusively by osteoblasts, promotes increased insulin secretion, pancreatic β-cell proliferation,

adiponectin secretion, and increases in energy expenditure (Lee *et al.*, 2007; Ng, 2011). Mice lacking the gene for osteocalcin are glucose intolerant and exhibit excess adiposity (Lee *et al.*, 2007), and treatment of mice with osteocalcin has shown beneficial effects on measures of glucose homeostasis. In mice fed a normal diet, injections of osteocalcin at 3 or 30 ng/g/day improved glucose tolerance and insulin sensitivity through increases in β-cell mass and insulin secretion. In mice fed a high-fat diet, injections of osetocalcin partially restored insulin sensitivity and glucose tolerance, and also resulted in increased energy expenditure and protection from diet-induced obesity (Ferron *et al.*, 2012), suggesting a role of osteocalcin in the improvement of insulin sensitivity and prevention of type 2 diabetes.

Associations between serum osteocalcin and insulin sensitivity may be observed in human subjects as well. In both men and postmenopausal women, serum osteocalcin is inversely associated with fasting plasma glucose, HbA_{1c}, and HOMA-IR, and is positively associated with β-cell function (Kanazawa *et al.*, 2011), suggesting that serum osteocalcin may positively influence glucose homeostasis by enhancing insulin action. *In vitro* and *ex vivo*, osteocalcin stimulates expression of adiponectin (Lee *et al.*, 2007), providing a mechanism by which osteocalcin may exert its insulin-sensitizing effects. However, a significant positive association of serum osteocalcin with improved glucose tolerance after adjusting for serum adiponectin levels was found in some (Kanazawa *et al.*, 2011; Shea *et al.*, 2009) but not all (Hwang *et al.*, 2012) studies utilizing human subjects, suggesting that osteocalcin-mediated increases in insulin sensitivity may involve mechanisms other than upregulation of adiponectin.

26.8 Conclusion

A large body of evidence strongly suggests a relationship between vitamin D and measures of insulin sensitivity (Figure 26.2). Epidemiological data shows a positive relationship between serum 25(OH)D and measures of insulin resistance, and in some studies treatment of individuals with $1\alpha,25(OH)_2D$ improves insulin resistance. Evidence suggests that the effects of vitamin D on insulin sensitivity occur at several levels, including through the stimulation of insulin secretion, by enhancing insulin signaling in target tissues, and through the regulation of additional hormones that affect glucose homeostasis. Because a positive relationship has been observed between vitamin D and muscle mass and function and skeletal muscle is recognized as the primary site of glucose disposal, it appears that increased insulin signaling and hypertrophy of muscle cells may explain some of the beneficial effects of vitamin D on whole-body glucose metabolism. Further identification of the mechanisms by which vitamin D exerts its insulin-sensitizing effects is necessary to establish recommendations for optimal serum levels of vitamin D for the management of glucose homeostasis and prevention of type 2 diabetes.

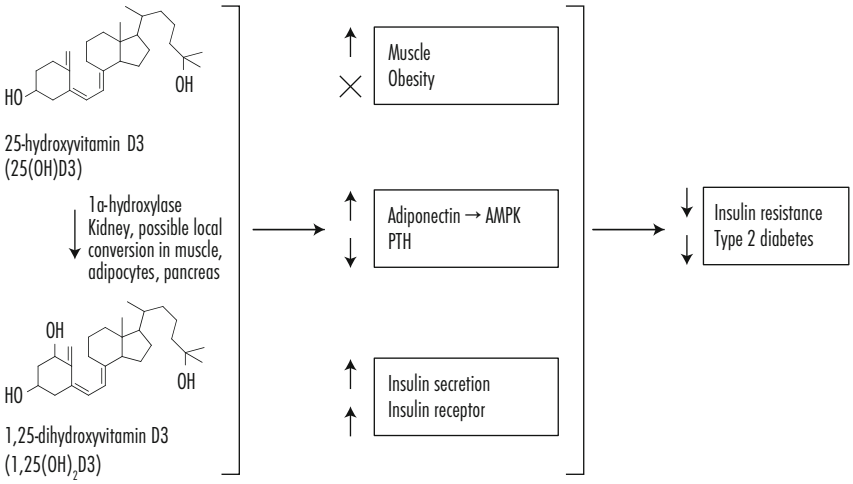

Figure 26.2. Potential mechanisms underlying the putative relationship between vitamin D and insulin sensitivity.

References

Alemzadeh, R., Kichler, J., Babar, G. and Calhoun, M., 2008. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. Metabolism Clinical and Experimental 57, 183-191.

Alvarez, J. and Ashraf, A., 2010. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homesostasis. International Journal of Endocrinology 2010, 351385.

Ardabili, H., Gargari, B. and Farzadi, L., 2012. Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. Nutrition Research 32, 195-201.

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoka, K., Kuriyama, H., Nishida, M., Yamashita, S., Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y., Funahashi, T. and Matsuzawa, Y., 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochemical and Biophysical Research Communications 257, 79-83.

Avruch, J., 1998. Insulin signal transduction through protein kinase cascades. Molecular and Cellular Biochemistry 182, 31-48.

Begum, N., Sussman, K. and Draznin, B., 1992. Calcium-induced inhibition of phosphoserine phosphatase in insulin target cells is mediated by the phosphorylation and activation of inhibitor 1. The Journal of Biological Chemistry 267, 5959-5963.

Bell, N., Epstein, S., Greene, A., Shary, J., Oexmann, M. and Shaw, S., 1985. Evidence for alteration of the vitamin D-endocrine system in obese subjects. Journal of Clinical Investigation 76, 370-373.

Bischoff-Ferrari, H., Dawson-Hughes, B., Willett, W., Staehelin, H., Bazemore, M., Zee, R. and Wong, J., 2004. Effect of vitamin D on falls: a meta-analysis. Journal of the American Medical Association 291, 1999-2006.

Bland, R., Markovic, D., Hills, C., Hughes, S., Chan, S., Squires, P. and Hewison, M., 2004. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. Journal of Steroid Biochemistry and Molecular Biology 89-90, 121-125.

Boden, G., 1997. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46, 3-10.

Boland, R., 2011. VDR activation of intracellular signaling pathways in skeletal muscle. Molecular and Cellular Endocrinology 347, 11-16.

Boland, R., Buitrago, C. and De Boland, A., 2005. Modulation of tyrosine phosphorylation signalling pathways by 1α,25$(OH)_2$-vitamin D3. Trends in Endocrinology and Metabolism 16, 280-287.

Bolland, M., Grey, A., Gamble, G. and Reid, I., 2005. Association between primary hyperparathyroidism and increased body weight: a meta-analysis. The Journal of Clinical Endocrinology & Metabolism 90, 1525-1530.

Bomfeldt, K. and Tabas, I., 2011. Insulin resistance, hyperglycemia, and atherosclerosis. Cell Metabolism 14, 575-585.

Bourlon, P., Billaudel, B. and Faure-Dussert, A., 1999. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. Journal of Endocrinology 160, 87-95.

Bourlon, P., Faure-Dussert, A., Billaudel, B., Sutter, B., Tramu, G. and Thomasset, M., 1996. Relationship between calbindin-D28K levels in the A and B cells of the rat endocrine pancreas and the secretion of insulin and glucagon: influence of vitamin D3 deficiency and 1,25-dihydroxyvitamin D3. Journal of Endocrinology 148, 223-232.

Brown, M. and Goldstein, J., 2008. Selective versus total insulin resistance: a pathogenic paradox. Cell Metabolism 7, 95-96.

Buitrago, C., Arango, N. and Boland, R., 2012. 1α,25$(OH)_2$D3-dependent modulation of Akt in proliferating and differentiating C2C12 skeletal muscle cells. Journal of Cellular Biochemistry 113, 1170-1181.

Buyukinan, M., Ozen, S., Kokkun, S. and Saz, E., 2012. The relation of vitamin D deficiency with puberty and insulin resistance in obese children and adolescents. Journal of Pediatric Endocrinology and Metabolism 25, 83-87.

Cade, C. and Norman, A., 1986. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat *in vivo*. Endocrinology 119, 84-90.

Chagas, C., Borges, M., Martini, L. and Rogero, M., 2012. Focus on vitamin D, inflammation, and type 2 diabetes. Nutrients 4, 52-67.

Chang, E., Donkin, S. and Teegarden, D., 2009. Parathyroid hormone supresses insulin signaling in adipocytes. Molecular and Cellular Endocrinology 307, 77-82.

Chiu, K., Chuang, L.-M., Lee, N., Ryu, J., McGullam, J., Tsai, G. and Saad, M., 2000. Insulin sensitivity is inversely correlated with plasma intact parathyroid hormone level. Metabolism 49, 1501-1505.

Choi, H., Willett, W., Stampfer, M., Rimm, E. and Hu, F., 2005. Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. Archives of Internal Medicine 165, 997-1003.

Chopra, I., Li, F., Wang, H. and Webster, K., 2012. Phosphorylation of the insulin receptor by AMP-activated protein kinase (AMPK) promotes ligand-independent activation of the insulin signalling pathway in rodent muscle. Diabetologia 55, 783-794.

D'Amour, P., 2012. Acute and chronic regulation of circulating PTH: significance in health and in disease. Clinical Biochemistry 45, 964-969.

De Boer, I., Tinker, L., Connelly, S., Curb, J., Howard, B., Kestenbaum, B., Larson, J., Manson, J., Margolis, K., Siscovick, D., Wiess, N. and Women's Health Initiative Investigators, 2008. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 31, 701-707.

DeLuca, H., 2004. Overview of general physiologic features and functions of vitamin D. The American Journal of Clinical Nutrition 80, 1689S-1696S.

DeLuca, H., 2008. Evolution of our understanding of vitamin D. Nutrition Reviews 66, S73-S87.

Dimopoulos, N., Watson, M., Sakamoto, K. and Hundal, H., 2006. Differential effects of palmitate and palmitoleate on insulin action and glucose utilization in rat L6 skeletal muscle cells. Biochemical Journal 399, 473-481.

Dirks-Naylor, A. and Lennon-Edwards, S., 2011. The effects of vitamin D on skeletal muscle function and cellular signaling. Journal of Steroid Biochemistry and Molecular Biology 125, 159-168.

Draznin, B., 1993. Cytosolic calcium and insulin resistance. American Journal of Kidney Diseases 21, 32-38.

Dzamko, N. and Steinberg, G., 2009. AMPK-dependent hormonal regulation of whole-body energy metabolism. Acta Physiologica 196, 115-127.

Erkelens, D., 2001. Insulin resistance syndrome and type 2 diabetes mellitus. The American Journal of Cardiology 88, 38J-42J.

Fantl, W., Johnson, D. and Williams, L., 1993. Signalling by receptor tyrosine kinases. Annual Review of Biochemistry 62, 453-481.

Ferron, M., McKee, M., Levine, R., Ducy, P. and Karsenty, G., 2012. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone 50, 568-575.

Flanagan, J., Wang, L., Tanqpricha, V., Reichrath, J., Chen, T. and Holick, M., 2003. Regulation of the 25-hydroxyvitamin D-1alpha-hydroxylase gene and its splice variant. Recent Results in Cancer Research 164, 157-167.

Ford, E., Ajani, U., McGuire, L. and Liu, S., 2005. Concentrations of serum vitamin D and the metabolic syndrome among US adults. Diabetes Care 28, 1228-1230.

Frayn, K., 2010. Metabolic regulation: a human perspective. Wiley-Blackwell, Chichester, UK, 384 pp.

Gannagé-Yared, M.-H., Chedid, R., Khalife, S., Azzi, E., Zoghbi, F. and Halaby, G., 2009. Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. European Journal of Endocrinology 160, 965-971.

Garcia, L., King, K., Ferrini, M., Norris, K. and Artaza, J., 2011. 1,25$(OH)_2$Vitamin D3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C_2C_{12} skeletal muscle cells. Endocrinology 152, 2976-2986.

George, P., Pearson, E. and Witham, M., 2012. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabetic Medicine 29, e142-e150.

Haffner, S., 1999. Epidemiology of insulin resistance and its relation to coronary artery disease. American Journal of Cardiology 84, 11J-14J.

Hardie, D., 2011. Sensing of energy and nutrients by AMP-activated protein kinase. The American Journal of Clinical Nutrition 93, 891S-896S.

Harkness, L. and Cromer, B., 2005. Low levels of 25-hydroxy vitamin D are associated with elevated parathyroid hormone in healthy adolescent females. Osteoporosis International 16, 109-113.

Hebert, S., Brown, E. and Harris, H., 1997. Role of the Ca^{2+}-sensing receptor in divalent mineral ion homeostasis. The Journal of Experimental Biology 200, 295-302.

Henriksen, E., Diamond-Stanic, M. and Marchionne, E., 2011. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radical Biology and Medicine 51, 993-999.

Hewison, M., Burke, F., Evans, K., Lammas, D., Sansom, D., Liu, P., Modlin, R. and Adams, J., 2007. Extra-renal 25-hydroxyvitamin D3-1α-hydroxylase in human health and disease. Journal of Steroid Biochemistry & Molecular Biology 103, 316-321.

Hirabara, S., Silveira, L., Abdulkader, F., Carvalho, C., Procopio, J. and Curi, R., 2007. Time-dependent effects of fatty acids on skeletal muscle metabolism. Journal of Cellular Physiology 210, 7-15.

Hurskainen, A-R., Virtanen, J., Tuomainen, T-P., Nurmi, T. and Voutilainen, S, 2012. Association of serum 25-hydroxyvitamin D with type 2 diabetes and markers of insulin resistance in a general older population in Finland. Diabetes/Metabolism Research and Reviews 28, 418-423.

Husemoen, L., Thuesen, B., Fenger, M., Jorgensen, T., Glumer, C., Svensson, J., Ovesen, L., Witte, D. and Linneberg, A., 2012. Serum 25(OH)D and type II diabetes association in a general population. Diabetes Care 35, 1695-1700.

Hwang, Y.-C., Jeong, I.-K., Ahn, K.-J. and Chung, H.-Y., 2012. Circulating osteocalcin level is associated with improved glucose tolerance, insulin secretion and sensitivity independent of the plasma adiponectin level. Osteoporosis International 23, 1337-1342.

Johnson, J., Grande, J., Roche, P. and Kumar, R., 1994. Immunohistochemical localization of the $1,25(OH)_2D3$ receptor and calbindin D_{28k} in human and rat pancreas. American Journal of Physiology 267, E356-360.

Jones, G., Strugnell, S. and DeLuca, H., 1998. Current understanding of the molecular actions of vitamin D. Physiological Reviews 78, 1193-1231.

Jové, M., Planavila, A., Sánchez, R., Merlos, M., Laguna, J. and Vázquez-Carrera, M., 2006. Palmitate induces tumor necrosis factor-α expression in C2C12 skeletal muscle cells by a mechanism involving protein kinase C and nuclear-factor κB activation. Endocrinology 147, 552-561.

Kadowaki, S. and Norman, A., 1984. Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. Journal of Clinical Investigation 73, 759-766.

Kahn, S., Hull, R. and Utzschneider, K., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444, 840-846.

Kanazawa, I., Yamaguchi, T., Tada, Y., Yamauchi, M., Yano, S. and Sugimoto, T., 2011. Serum osteocalcin level is positively associated with insulin sensitivity and secretion in patients with type 2 diabetes. Bone 48, 720-725.

Kaufman, F. and Shaw, J., 2007. Type 2 diabetes in youth: rates, antecedents, treatment, problems and prevention. Pediatric Diabetes 8, 4-6.

Kawashima, H. and Castro, A., 1981. Effect of 1 alpha-hydroxyvitamin D3 on the glucose and calcium metabolism in genetic obese mice. Research Communications in Chemical Pathology Pharmacology 33, 155-161.

Kishida, K., Funahashi, T. and Shimomura, I., 2012. Molecular mechanisms of diabetes and atherosclerosis: role of adiponectin. Endocrine, Metabolic & Immune Disorders – Drug Targets 12, 118-131.

Kumar, S., Olukoga, A., Gordon, C., Mawer, E., France, M., Hosker, J., Davies, M. and Boulton, A., 1994. Impaired glucose tolerance and insulin sensitivity in primary hyperparathyroidism. Clinical Endocrinology (Oxf) 40, 47-53.

Lee, N., Sowa, H., Hinoi, E., Ferron, M., Ahn, J., Confavreux, C., Dacquin, R., Mee, P., McKee, M., Jung, D., Zhang, Z., Kim, J., Mauvais-Jarvis, F., Ducy, P. and Karsenty, G., 2007. Endocrine regulation of energy metabolism by the skeleton. Cell 130, 456-469.

Li, J., Byrne, M., Chang, E., Jiang, Y., Donkin, S, Buhman, K., Burgess, J. and Teegarden, D., 2008. 1α,25-dihydroxyvitamin D hydroxylase in adipocytes. Journal of Steroid Biochemistry and Molecular Biology 112, 122-126.

Li, Z., Joyal, J. and Sacks, D., 2000. Binding of IRS proteins to calmodulin is enhanced in insulin resistance. Biochemistry 39, 5089-5096.

Lizunov, V., Matsumoto, H., Zimmerberg, J., Cushman, S. and Frolov, V., 2005. Insulin stimulates the halting, tethering, and fusion of mobile GLUT4 vesicles in rat adipose cells. The Journal of Cell Biology 169, 481-489.

Lizunov, V., Stenkula, K., Lisinski, I., Gavrilova, O., Yver, D., Chadt, A., Al-Hasani, H., Zimmerberg, J. and Cushman, S., 2012. Insulin stimulates fusion, but not tethering, of GLUT4 vesicles in skeletal muscle of HA-GLUT4-GFP transgenic mice. American Journal of Physiology 302, E950-E960.

Lowell, B. and Shulman, G., 2005. Mitochondrial dysfunction and type 2 diabetes. Science 307, 384-387.

Maestro, B., Campión, J., Dávila, N. and Calle, C., 2000. Stimulation by 1,25-dihydroxyvitamin D3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells. Endocrine Journal 47, 383-391.

Maestro, B., Dávila, N., Carranza, C. and Calle, C., 2003. Identification of a vitamin D response element in the human insulin receptor gene promoter. Journal of Steroid Biochemistry & Molecular Biology 84, 223-230.

Maestro, B., Molero, S., Bajo, S., Dávila, N. and Calle, C., 2002. Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvitamin D3. Cell Biochemistry and Function 20, 227-232.

Mak, R., 1998. 1,25-Dihydroxyvitamin D3 corrects insulin and lipid abnormalities in uremia. Kidney International 53, 1353-1357.

Marantes, I., Achenbach, S., Atkinson, E., Khosla, S., Melton, L. and Amin, S., 2011. Is vitamin D a determinant of muscle mass and strength? Journal of Bone and Mineral Research 26, 2860-2871.

McCarty, M., 2006. PKC-mediated modulation of L-type calcium channels may contribute to fat-induced insulin resistance. Medical Hypotheses 66, 824-831.

Mitri, J., Muraru, M. and Pittas, A., 2011. Vitamin D and type 2 diabetes: A systematic review. European Journal of Clinical Nutrition 65, 1005-1015.

Mora, S. and Pessin, J., 2002. An adipocentric view of signaling and intracellular trafficking. Diabetes/Metabolism Research and Reviews 18, 345-356.

Mozaffari-Khosravi, H., Hosseinzadeh-Shamsi-Anar, M., Salami, M.-A., Hadinedoushan, H. and Mozayan, M., 2012. Effects of a single post-partum injection of a high dose of vitamin D on glucose tolerance and insulin resistance in mothers with first-time gestational diabetes mellitus. Diabetic Medicine 29, 36-42.

Muir, S. and Montero-Odasso, M., 2011. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. Journal of the American Geriatric Association 59, 2291-2300.

Muniyappa, R., Lee, S., Chen, H. and Quon, M., 2008. Current approaches for assessing insulin sensitivity and resistance *in vivo*: advantages, limitations, and appropriate usage. American Journal of Physiology 294, E15-E26.

Muoio, D. and Newgard, C., 2008. Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nature Reviews Molecular Cell Biology 9, 193-205.

Need, A., O'Loughlin, P., Horowitz, M. and Nordin, B., 2005. Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. Clinical Endocrinology 62, 738-741.

Neyestani, T., Nikooyeh, B., Alavi-Majd, H., Shariatzadeh, N., Kalayi, A., Tayebinejad, N., Heravifard, S., Salekzamani, S. and Zahedirad, M., 2012. Improvement of vitamin D status via daily intake of fortified yogurt drink either with or without extra calcium ameliorates systemic inflammatory biomarkers, including adipokines, in the subjects with type 2 diabetes. Journal of Clinical Endocrinology & Metabolism 97, 2005-2011.

Ng, K., 2011. Regulation of glucose metabolism and the skeleton. Clinical Endocrinology 75, 147-155.

Ni, Z., Smogorzewski, M. and Massry, S., 1994. Effects of parathyroid hormone on cytosolic calcium of rat adipocytes. Endocrinology 135, 1837-1844.

Nimitphong, H., Chanprasertyothin, S., Jongjaroenprasert, W. and Ongphiphadhanakul B, 2009. The association between vitamin D status and circulating adiponectin independent of adiposity in subjects with abnormal glucose tolerance. Endocrinology 36, 205-210.

Olson, M., Maalouf, N., Oden, J., White, P. and Hutchison, M., 2012. Vitamin D deficiency in obese children and its relationship to glucose homeostasis. Journal of Clinical Endocrinology and Metabolism 97, 279-285.

Orwoll, E., Riddle, M. and Prince, M., 1994. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. American Journal of Clinical Nutrition 59, 1083-1087.

Palomer, X., González-Clemente, J., Blanco-Vaca, F. and Mauricio, D., 2008. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes, Obesity and Metabolism 10, 185-197.

Pepe, J., Romagnoli, E., Nofroni, I., Pacitti, M., De Geronimo, S., Letizia, C., Tonnarini, G., Scarpiello, A., D'Erasmo, E. and Minisola, S., 2005. Vitamin D status as the major factor determining the circulating levels of parathyroid hormone: a study in normal subjects. Osteoporosis International 16, 805-812.

Perna, A., Fadda, G., Zhou, X.-J. and Massry, S., 1990. Mechanisms of impaired insulin secretion after chronic excess of parathyroid hormone. American Journal of Physiology 259, F210-F216.

Pfeifer, M., Begerow, B. and Minne, H., 2002. Vitamin D and muscle function. Osteoporosis International 13, 187-194.

Pittas, A., Harris, S., Stark, P. and Dawson-Hughes, B., 2007b. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 30, 980-986.

Pittas, A., Lau, J., Hu, F. and Dawson-Hughes, B., 2007a. Review: the role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. The Journal of Clinical Endocrinology and Metabolism 92, 2017-2029.

Prentki, M. and Nolan, C., 2006. Islet β cell failure in type 2 diabetes. The Journal of Clinical Investigation 116, 1802-1812.

Rajakumar, K., Fernstrom, J., Holick, M., Janosky, J. and Greenspan, S., 2008. Vitamin D status and response to vitamin D3 in obese vs. non-obese African American children. Obesity 16, 90-95.

Reaven, G., 2005a. The insulin resistance syndrome: definition and dietary approaches to treatment. Annual Reviews in Nutrition 25, 391-406.

Reaven, G., 2005b. Insulin resistance, the insulin resistance syndrome, and cardiovascular disease. Panminerva Medica 47, 201-210.

Reis, J., Von Mühlen, D., Kritz-Silverstein, D., Wingard, D., Barrett-Connor, E., 2007. Vitamin D, parathyroid hormone levels, and the prevalence of metabolic syndrome in community-dwelling older adults. Diabetes Care 30, 1549-1555.

Resnick, L., 1992. Cellular ions in hypertension, insulin resistance, obesity, and diabetes: a unifying theme. Journal of the American Society of Nephrology 3, S78-S85.

Reusch, J., Begum, N., Sussman, K. and Draznin, B., 1991. Regulation of GLUT-4 phosphorylation by intracellular calcium in adipocytes. Endocrinology 129, 3269-3273.

Roth, C., Elfers, C., Kratz, M. and Hoofnagle, A., 2011. Vitamin D deficiency in obese children and its relationship to insulin resistance and adipokines. Journal of Obesity 2011, 495101.

Schmitz-Peiffer, C., Craig, D. and Biden, T., 1999. Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. The Journal of Biological Chemistry 274, 24202-24210.

Schwanstecher, C. and Schwanstecher, M., 2011. Targeting type 2 diabetes. Handbook of Experimental Pharmacology 203, 1-33.

Scragg, R., Sowers, M. and Bell, C., 2004. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 27, 2813-2818.

Shankar, A., Sabanayagam, C. and Kalidindi, S., 2011. Serum 25-hydroxyvitamin D levels and prediabetes among subjects free of diabetes. Diabetes Care 34, 1114-1119.

Shea, M., Gundberg, C., Meigs, J., Dallal, G., Saltzman, E., Yoshida, M., Jacques, P. and Booth, S., 2009. Gamma-carboxylation of osteocalcin and insulin resistance in older men and women. American Journal of Clinical Nutrition 90, 1230-1235.

Shin, Y., Kim, K., Lee, C., Shin, H., Kang, M., Lee, H-R. and Lee, Y-J., 2012. High prevalence of vitamin D insufficiency or deficiency in young adolescents in Korea. European Journal of Pediatrics 171(10), 1475-1480.

Siddiqui, S., Chang, E., Li, J., Burlage, C., Zou, M., Buhman, K., Koser, S., Donkin, S. and Teegarden, D., 2008. Dietary intervention with vitamin D, calcium, and whey protein reduced fat mass and increased lean mass in rats. Nutrition Research 28, 783-790.

Snijder, M., Van Dam, R., Visser, M., Deeg, D., Dekker, J., Bouter, L., Seidell, J. and Lips, P., 2005. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. The Journal of Clincial Endocrinology & Metabolism 90, 4119-4123.

Sun, X., Crimmins, D., Myers, M., Miralpeix, M. and White, M., 1993. Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. Molecular and Cellular Biology 13, 7418-7428.

Takiishi, T., Gysemans, C., Bouillon, R. and Mathieu, C., 2012. Vitamin D and diabetes. Rheumatic Disease Clinics of North America 38, 179-206.

Taniguchi, C., Emanuelli, B. and Kahn, R, 2006. Critical nodes in signaling pathways: insights into insulin action. Nature Reviews Molecular Cell Biology 7, 85-96.

Teegarden, D. and Donkin, S., 2009. Vitamin D: emerging new roles in insulin sensitivity. Nutrition Research Reviews 22, 82-92.

Tishinsky, J., Robinson, L. and Dyck, D., 2012. Insulin-sensitizing properties of adiponectin. Biochimie 94(10), 2131-2136.

Towler, M. and Hardie, D., 2007. AMP-activated protein kinase in metabolic control and insulin signaling. Circulation Research 100, 328-341.

Ullrich, A. and Schlessinger, J., 1990. Signal transduction by receptors with tyrosine kinase activity. Cell 61, 203-212.

Vaidya, A., Williams, J. and Forman, J., 2011. The independent association between 25-hydroxyvitamin D and adiponectin and its relation with BMI in two large cohorts: the NHS and the HPFS. Obesity 20, 186-191.

Viollet, B., Andreelli, F., Jørgensen, S., Perrin, C., Geloen, A., Flamez, D., Mu, J., Lenzer, C., Baud, O., Bennoun, M., Gomas, E., Nicholas, G., Wojtaszewski, J., Kahn, A., Carling, D., Schuit, F., Birnbaum, M., Richter, E., Burcelin, R. and Vaulont, S., 2003. The AMP-activated protein kinase α2 catalytic subunit controls whole-body insulin sensitivity. Journal of Clinical Investigation 111, 91-98.

Von Hurst, P., Stonehouse, W. and Coad, J., 2010. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial. British Journal of Nutrition 103, 549-555.

Weigert, C., Kron, M., Kalbacher, H., Pohl, A., Runge, H., Häring, H., Schleicher, E. and Lehmann, R., 2008. Interplay and effects of temporal changes in the phosphorylation state of serine-302, -307, and -318 of insulin receptor substrate-1 on insulin action in skeletal muscle cells. Molecular Endocrinology 22, 2729-2740.

Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R. and Tataranni, A., 2001. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. The Journal of Clinical Endocrinology & Metabolism 86, 1930-1935.

White, M., 2002. IRS proteins and the common path to diabetes. American Journal of Physiology 283, E413-E422.

Winder, W. and Hardie, D., 1996. Inactivation of acety-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. American Journal of Physiology 270, E299-E304.

Wu, T., Willett, W. and Giovannucci, E., 2009. Plasma C-peptide is inversely associated with calcium intake in women and with 25-hydroxy vitamin D in men. The Journal of Nutrition 139, 547-554.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P. and Kadowaki, T., 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature Medicine 7, 941-946.

Young, M., Schwartz, G., Wang, L., Jamieson, D., Whitlatch, L., Flanagan, J., Lokeshwar, B., Holick, M. and Chen, T., 2004. The prostate 25-hydroxyvitamin D-1α-hydroxylase is not influenced by parathyroid hormone and calcium: implications for prostate cancer chemoprevention by vitamin D. Carcinogenesis 25, 967-971.

Zehnder, D., Bland, R., Williams, M., McNinch, R., Howie, A., Stewart, P. and Hewison, M., 2001. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. The Journal of Clinical Endocrinology & Metabolism 86, 888-894.

Zeitz, U., Weber, K., Soegiarto, D., Wolf, E., Balling, R. and Erben, R., 2003. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. The FASEB Journal 17, 509-511.

Zheng, D., MacLean, P., Pohnert, S., Knight, J., Olson, A., Winder, W. and Dohm, L., 2001. Regulation of muscle GLUT-4 transcription by AMP-activated protein kinase. Journal of Applied Physiology 91, 1073-1083.

Zhou, Q., Hou, F., Guo, Z., Liang, M., Wang, G. and Zhang, X., 2008. 1,25-dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. Diabetes/Metabolism Research and Reviews 24, 459-464.

Zierold, C., Mings, J. and DeLuca, H., 2003. Regulation of 25-hydroxyvitamin D3-24-hydroxylase mRNA by 1,25-dihydroxyvitamin D3 and parathyroid hormone. Journal of Cellular Biochemistry 88, 234-237.

Key facts

- Diabetes is a metabolic disease, in which a person has high blood sugar resulting from inadequate production and/or utilization of insulin. Its classical symptoms include polyuria, polydipsia, weight loss, and/or polyphagia.
- Three main types of diabetes: Type 1 diabetes mellitus (T1D), predominately observed among children and youth; Type 2 diabetes mellitus (T2D), mainly occurred among adults; and gestational diabetes mellitus (GDM), among women who had no diabetes before, but with a high blood glucose level during pregnancy.
- Globally, the prevalence rate of diabetes is continually increasing and, by 2030, the number of people with diabetes will reach to 366 million with a noticeable increase from the developing countries.
- Vitamin D, a necessity for maintaining bone health, may have been involved in a wide range of biological actions due to that vitamin D receptor is present in most tissues and cells in the body.
- Laboratory studies have suggested the role of vitamin D in insulin synthesis and secretion, but a causal relationship between vitamin D supplements and diabetes prevention has not been established yet from clinical trials.

Summary points

- Many studies have observed a reverse relationship between serum vitamin D levels and the risk of diabetes, but how vitamin D plays a physiologically important role in the development of diabetes is unclear.
- T1D is believed as the results of autoimmune destruction of insulin-producing beta cells of the pancreas. However, the exact mechanism is not totally understood.
- A seasonal pattern of the incidence of T1D has been observed by many epidemiological studies in different countries, regions, and ethnicity groups. The incidence of T1D seems to peak more in winter seasons.
- T2D is originally described as a lifelong disease mainly occurring among elderly adults, but it is now observed to affect younger adults and even children.
- The results from interventional studies in examining whether vitamin D supplements reduce T2D risk are mixed, but no study has reported that taking vitamin D supplement will worsen the pathology of the disease.
- The prevalence rates of GDM vary by region, but are not affected by latitude. However, vitamin D deficiency seems very prevalent in pregnant women.
- Adult stature components (e.g. standing height, leg length, ratio of leg length to standing height, and femur bone length) are observed to be negatively associated with risk of T2D.
- Emerging evidence indicates that bones, the target organ of vitamin D, have an endocrine function. Metabolic molecules of bone modeling may play an important role in the development of diabetes.

27. Vitamin D and diabetes mellitus: where are we?

J. Liu
Department of Community Health Sciences, Brock University, 500 Glenridge Ave. St. Catharines, ON L2S 3A1, Canada; jliu@brocku.ca

Abstract

Diabetes mellitus, as a common metabolic disease, affects millions people's health worldwide and its incidence is continuing to increase. It is important to find effective measures of prevention and/or intervention for the disease to reduce its impact on the risk for cardiovascular disease – the leading cause of mortality among patients with diabetes mellitus. Apart from its well-known 'classic' effect in bone metabolism, vitamin D has received widespread attention for its potential role in the prevention of diabetes mellitus, in particular type 2 diabetes mellitus. A large number of studies have observed a reverse relationship between vitamin D levels and the risk of diabetes mellitus. The results from the studies aiming to reduce risk for diabetes mellitus with vitamin D supplements are mixed though no study reports worsening the disease pathologically after treatment. Most previous studies on the relationship between vitamin D and diabetes mellitus were orientated from the direction of the 'non-classic' effect of vitamin D, but the emerging evidence indicates that its 'classic' effect on bone metabolism can be the key to understand its risk association with diabetes mellitus.

Keywords: diabetes mellitus, bone metabolism

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_27, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-dihydroxyvitamin D
GDM	Gestational diabetes mellitus
NOD	Nonobese diabetic
RCT	Randomized controlled trial
RECORD	Rosiglitazone evaluated for cardiac outcomes and regulation of glycaemia in diabetes
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
VDR	Vitamin D receptor
WHI	Women's Health Initiative

27.1 Introduction

27.1.1 Vitamin D

Vitamin D is well known for its primary physiological role of regulation of calcium homeostasis in maintaining bone health. However, growing evidence indicates that vitamin D is also involved in modulating body composition, energy homeostasis, insulin sensitivity, and immune function (Holick *et al.*, 2011; Mithal *et al.*, 2009; Wolden-Kirk *et al.*, 2011); thus, vitamin D deficiency has been observed to be associated with a broadening field of health problems including certain cancers, cardiovascular disease, and diabetes mellitus (Anderson *et al.*, 2010; Garland *et al.*, 1989, 1990; Pittas *et al.*, 2007).

There are two sources of vitamin D available for the human body; endogenous synthesis in the skin from exposure to sunlight and exogenous consumption in foods and/or pharmaceutical supplements. The endogenously synthesised vitamin D comprises approximately 80-90% of circulating levels of vitamin D (Mithal *et al.*, 2009). As expected, season and latitude affect the levels of vitamin D (Mithal *et al.*, 2009; Pettifor *et al.*, 1996; Van der Mei *et al.*, 2007).

The active metabolite of vitamin D in the human body is $1,25(OH)_2D$, which is generated in multiple steps. 25(OH)D is produced first in the liver through D-25 hydroxylase and then further converted to $1,25(OH)_2D$, primarily but not exclusively, in the kidneys by the 25(OH)D-1α-hydroxylases (Holick, 2009). Within the circulation, vitamin D-binding protein is the major transport protein for vitamin D metabolites in plasma. $1,25(OH)_2D$ can bind to the intracellular vitamin D nuclear receptors, which are present in most tissues and cells of the body(Adams and Hewison, 2010; Holick, 2007), and then initiate the activations that regulate not only calcium metabolism, but also differentiation and division of various cell types (Nagpal *et al.*, 2005). Although $1,25(OH)_2D$ is the biologically active form of vitamin D, however, serum 25(OH)D

concentrations are recommended as the best indicator in reflecting vitamin D levels from either exposure to sunlight or dietary sources (Holick, 2009).

The primarily biological role of vitamin D is known for its regulating function of calcium metabolism to maintain extracellular calcium ion levels within a physiologically acceptable range. Since the physiologically acceptable range of calcium is very tight (8.5-10.2 mg/dl) and the concentration of calcium distribution between outside and inside of the cell is balanced at a vast difference ratio (10,000:1), any change that occurs to the range or the balance will cause various disease sequelae (Fujita, 2000). Extreme vitamin D deficiency will result in rickets in children and osteomalacia in adults; while vitamin D deficiency in certain degrees will increase the risk for many metabolic diseases, particularly for diabetes mellitus (Adams and Hewison, 2010). The VDR has been found to be expressed in beta cells in pancreatic and other vitamin D related genes such as vitamin D-binding protein and D-24-hydroxylase, which degrades the active form of vitamin D into water-soluble inactive forms (Bikle, 2009). Although many epidemiological studies and animal experimental studies have observed a reverse relationship between serum vitamin D levels and the risk of diabetes mellitus (see details below), it is not totally understood how vitamin D signaling plays a physiologically important role in beta cells and in the development of diabetes mellitus.

27.1.2 Diabetes mellitus

The increasing prevalence of diabetes mellitus is rising at an alarming rate globally. It is estimated that approximately 346 million people worldwide have diabetes and the incidence is continuing to increase (WHO, 2011a). Diabetes mellitus significantly increases the risk for coronary heart disease, stroke, renal failure, lower limb amputations, and visual impairment and blindness. People with diabetes mellitus require at least two to three times higher the health-care resources, which may account for up to 15% of national health care budgets (WHO, 2011b). Three main types of diabetes mellitus are recognized, i.e. T1D, T2D and GDM. The vast majority of the diabetic patients (90-95%) are with type 2 diabetes.

Type 1 diabetes mellitus

T1D, previously known as juvenile diabetes or insulin-dependent diabetes mellitus, is usually diagnosed in children and younger adults. The incidence of T1D is increasing in children and youth by about 3% (range 2-5%) per annum with approximately 76,000 children aged less than 15 years annually developing T1D worldwide (IDF, 2009). The increase has been observed in countries with both high and low prevalence, particularly with a steeper increase in some of the low-prevalence countries (Aanstoot *et al.*, 2007). The incidence rates of T1D vary between countries with countries such as China reporting the lowest incidence rates (about 0.57 cases per 100,000 population younger than 18 years of age per year) to rates roughly 30 times higher in the UK (18-20 per 100,000 per year) to almost 100-fold higher (about 48-49 per 100,000 per year) in Finland and Sardinia (Daneman, 2006). T1D is seen predominantly in children younger than 15~ years of age with a significant trend towards decreasing age at presentation (IDF, 2009).

Individuals with T1D do not produce insulin in their body, which is believed to be the result of autoimmune processes between genetic and environmental factors. The variation of human leukocyte antigen genes is considered to contribute approximately 50% of the total genetic susceptibility to T1D (Risch, 1987). Pathologically, T1D is a condition in which pancreatic cell destruction leads to absolute insulin deficiency. Two subtypes have been identified in T1D; type 1A, which includes the common, immune-mediated forms of the disease (Concannon *et al.*, 2009; Devendra *et al.*, 2004); and type 1B, which is far less frequent, has usually unknown causes, and occurs mostly in individuals of Asian or African descent who have varying degrees of insulin deficiency between sporadic episodes of ketoacidosis (Abiru *et al.*, 2002). The exact mechanism of the disease is not totally understood, but the consensus is that the majority of the patients with T1D acquire the disease as a result of autoimmune destruction of insulin-producing beta cells of the pancreas (Concannon *et al.*, 2009; Daneman, 2006). It is believed that several genetic and environmental risk factors are involved in the development of T1D.

Type 2 diabetes mellitus

T2D prevalence has been increasing dramatically worldwide in both genders during last three decades, but this increase is more dramatically seen in women (the prevalence of diabetes mellitus for women was increased 23% from 7.5% in 1980 to 9.2% in 2008; and for men by 18% from 8.3% in 1980 to 9.8% in 2008) (Danaei *et al.*, 2011). Although originally described as a lifelong disease mainly occurring among the elderly adults, T2D (also known as adult-onset diabetes or non-insulin-dependent diabetes mellitus) is now observed to affect younger adults and even children. With the rise of overweight and obese individuals and the changing of life style, concern has risen about a global T2D epidemic (Zimmet *et al.*, 2001). It has been projected that the number of people with diabetes worldwide will increase from 171 million in 2000 to 366 million by 2030 with a noticeable increase from the developing countries. For instance, the number of people with T2D in China will have more than doubled from 20.7 million in 2000 to 42.3 million in 2030, which is a much faster rate than that in United States and Canada (19.7 million in 2000 to 33.9 million in 2030) (Hossain *et al.*, 2007). Individuals with T2D usually have adverse lipid profiles and are at a significant risk of increased mortality from cardiovascular disease (Liu *et al.*, 2005).

In contrast to T1D, people with T2D are believed to have insulin insensitivity, initially with compensatory increase in insulin secretion, but the beta-cells in the pancreas may fail to keep up with the increased workload resulting in a relative insulin insufficiency and eventually the development of T2D. Although insulin insensitivity and beta-cell dysfunction are critical to the development of impaired glucose tolerance and T2D, the exact mechanisms are complicated and not well understood. There is a genetic basis for the dysfunction in both parameters, but environmental factors are undoubtedly to play a major role in its process. Vitamin D deficiency has been suggested to be one of the environmental factors and its relationship with the risk of T2D has been primarily investigated by a number of studies in humans (Osei, 2010).

Gestational diabetes mellitus

GDM is hyperglycaemia with onset or first recognition during pregnancy. Although the symptoms of GDM are similar to T2D, it is often diagnosed through prenatal screening, rather than reported symptoms. Women with GDM are at a great increased risk of developing T2D in comparison to those women without GDM (Bellamy *et al.*, 2009).

Since lack of uniform standards in glucose tolerance testing for pregnant women in many countries there is no an accurate estimation of the global incidence of GDM (Ben-Haroush *et al.*, 2004). The prevalence rates estimated in certain areas, such as Europe and China, were between 2.0 and 6.0% (Buckley *et al.*, 2011; Zhang *et al.*, 2011). However, an international multicenter study of GDM conducted in a heterogeneous, multinational, multicultural, and ethnically diverse cohort with approximately 25,000 women in the third trimester of gestation found that about 18% (range 9.3-25.5%) of the pregnancies were affected by GDM after performing a 75-g oral glucose tolerance test (Sacks *et al.*, 2012). It has been observed that GDM is more frequent in certain ethnic groups than in the general population. In general, white women have a lower incidence than black women; while Asian women have the highest rate (Reece *et al.*, 2009).

Although usually an increase in the concentration of pregnancy hormones leads to a change of metabolism pattern of the body and may reduce tissues sensitivity to insulin, for some women pregnancy is a trigger for a series of metabolic imbalances that lead to a diabetic state. These women may have already been genetically vulnerable. In addition, when environmental factors, such as diet, obesity, depression, etc. are present these women may increase the likelihood of developing diabetes (Lain and Catalano, 2007). It has also been suggested that vitamin D deficiency may play a role for the occurrence of GDM (Dror, 2011).

27.2 Vitamin D deficiency and diabetes mellitus

27.2.1 Vitamin D deficiency definition

Currently, no standard definition of optimal vitamin D status exists and there is a considerable controversy on the definition of vitamin D deficiency. The recently updated position statement from the Institutes of Medicine suggests that vitamin D as serum 25(OH)D with levels less than 30 nmol/l (12 ng/ml) is considered as deficiency; and less than 50 nmol/l (20 ng/ml) but higher than 30 nmol/l as inadequacy in regard to bone health (Ross *et al.*, 2011). However, many others believe that a much higher level of vitamin D may be needed to maintain overall health beyond just bone health (Holick *et al.*, 2011), particularly when examining vitamin D levels and its risk association with diabetes mellitus and other chronic diseases. There is, currently, consideration of the widespread prevalence of hypovitaminosis D, as it may affect people worldwide, particularly the children and the elderly (Holick *et al.*, 2011; Mithal *et al.*, 2009).

27.2.2 Vitamin D deficiency and type 1 diabetes mellitus

The seasonal pattern of the incidence of T1D has been observed by many epidemiological studies in different countries, regions, and ethnicity groups (Fleegler *et al.*, 1978; Kahn and Anderson, 2009; Moltchanova *et al.*, 2009; Weets *et al.*, 2006). The incidence of T1D seems to peak more in the winter season than other seasons in many countries located in both the northern and the southern hemispheres (Moltchanova *et al.*, 2009). Since the primary source of circulating vitamin D in humans is largely derived from processes initiated by ultraviolet radiation exposure, which is in nature inversely associated with latitude, several studies have reported a latitudinal gradient for the prevalence of T1D (Anonymus, 1988; 2000; Staples *et al.*, 2003). In Newfoundland, Canada, one of the highest documented incidences of T1D worldwide, the results of ecological analysis have suggested a link between T1D risk and limited UVB exposure (Sloka *et al.*, 2010). Data from 51 regions worldwide was used to examine the relationship between UVB irradiance and age-standardised incidence rates of T1D in children and indicated that the incidence of T1D was greater at higher latitudes (Mohr *et al.*, 2008). Many observational studies have also showed that a lower level of serum vitamin D or vitamin D deficiency is very common among those newly diagnosed T1D patients in children, adolescents, and young adults (Borkar *et al.*, 2010; Cooper *et al.*, 2011; Littorin *et al.*, 2006; Pozzilli *et al.*, 2005; Svoren *et al.*, 2009).

NOD mice have been used as models in animal experimental studies to examine vitamin D deficiency and the risk of diabetes. The results from most of the studies, except for one (Hawa *et al.*, 2004), supported what has been observed from human's studies. NOD mice that are genetically predisposed to develop insulitis and T1D had the disease developed earlier when growing in a vitamin D depleted environment (Giulietti *et al.*, 2004). When the active metabolites of vitamin D were administrated in early life of these mice, it significantly decreased the risk of developing T1D (Mathieu *et al.*, 1994; Zella *et al.*, 2003). Those treated with vitamin D had much higher levels of serum calcium (Zella *et al.*, 2003). The results from mice lacking VDR seemed to be more complicated. In comparing to NOD mice without VDR knockout, those VDR knockout NOD mice were not different in glucose homeostasis and incidence of diabetes (Cheng *et al.*, 2011; Gysemans *et al.*, 2008). However, those VDR knockout mice significantly increased mRNA expression and protein production from the local pancreatic islet renin-angiotensin system; while pre-treatment with 1,25(OH) could prevent and reverse the increase in the renin-angiotensin system component formation and improve insulin secretion. This formation is induced by high glucose concentrations in isolated mouse islets (Cheng *et al.*, 2011). Furthermore, when those VDR knockout mice were fed a standard diet, the capacity of islet-cells to produce and/or secrete insulin was severely impaired by the hypocalcaemia, but it could be normalized with feeding a high-lactose calcium rescue diet (Driver *et al.*, 2011). This suggests that the effect of vitamin D on diabetes may, at least in part, be mediated through the role of calcium.

Results from several cohort or population-based case-control studies with vitamin D supplements supported the link between vitamin D deficiency and the risk of T1D, though some others did not. The results from a birth-cohort study of northern Finland indicated that taking vitamin D in early life could reduce the risk for T1D in later life. Approximately 12,000 Finnish children enrolled at a

1966 baseline. During an approximately 30 years follow-up, it was found that those children with suspected rickets during their first year of life had a 3-times higher risk of developing T1D later in life; while those who received at least 2,000 IU/day of vitamin D during their first year of life reduced risk of developing T1D later in life by 88% (Hypponen *et al.*, 2001). A case-control study from a nested cohort conducted in Norway found that cod liver oil supplementation in infancy or during maternal pregnancy was associated with a lower risk of T1D (Stene and Joner, 2003; Stene *et al.*, 2000); and, further, that children from mothers with lower levels of serum 25(OH)D during pregnancy were at increased risk of T1D (Sorensen *et al.*, 2011). However, the results from two other studies showed that either maternal intake of vitamin D during pregnancy (Marjamaki *et al.*, 2010) or during infancy did not affect the risk of T1D (Simpson *et al.*, 2011), though both of these studies had no information on the fetal and/or maternal level serum 25(OH)D.

The distribution of the VDR gene and its relationship with T1D in human has also been examined by several studies though the results are inconsistent. For instance, a recently case-control study conducted in a group of Turkish patients with T1D found that bone turnover markers, such as osteocalcin and C telopeptide, and bone mineral density were significantly lower in patients with T1D, but that the VDR gene polymorphisms, Bsm1, Fok 1, Apa1, and Taq1 showed no difference between those cases and controls (Gogas Yavuz *et al.*, 2011). A similar result has been reported from a previous meta-analysis, which showed no association between VDR gene polymorphisms and T1D risk (Guo *et al.*, 2006). However, another meta-analysis conducted with the similar data suggested that the risk association of the VDR polymorphisms genes with T1D might be affected by the levels of winter ultraviolet radiation. Therefore, the involvement of VDR variants in the etiology of T1D could not be excluded (Ponsonby *et al.*, 2008).

27.2.3 Vitamin D deficiency and type 2 diabetes mellitus

The seasonal pattern for the risk of T2D does not appear as that for T1D, though a few of studies have shown seasonal variations of concentrations of pre-prandial glucose and HbA_{1c} in T2D patients with levels higher in winter and lower in summer (Ishii *et al.*, 2001; Liang, 2007).

However, a number of observational studies have shown a reversed risk association of T2D with vitamin D in the relationship between higher levels of intake vitamin D and/or calcium and a lower T2D risk or that between lower levels of serum 25(OH) D and an increased risk for incident T2D (Kirii *et al.*, 2009; Knekt *et al.*, 2008; Pittas *et al.*, 2006; Scragg *et al.*, 1995, 2004). However, the results from some other studies were not in favor of this link (Grimnes *et al.*, 2010; Pittas *et al.*, 2010; Robinson *et al.*, 2010). For instance, lower serum 25(OH) levels might be associated with the increased risk of incident T2D in men(Knekt *et al.*, 2008), but not in old women (Robinson *et al.*, 2010). The ethnicity background seems to affect this association too; a reversed relationship between serum 25(OH)D and risk of T2D has been observed in New Zealand Polynesians and Caucasians, but those Polynesians had much lower levels of 25(OH)D (Scragg *et al.*, 1995). Similarly, results from an analysis of the third national health and nutrition examination survey showed a reversed relationship between serum 25(OH)D levels and T2D risk among Mexican-Americans and Hispanic Whites, but not in non-Hispanic blacks (Scragg *et*

al., 2004). The results from another analysis conducted among middle-aged Caucasian men and women indicated that individuals with serum 25(OH)D levels ≥80 nmol/l were half as likely to have diabetes in comparison to those with levels <37 nmol/l (Brock *et al.*, 2011).

In addition, central adiposity status is considered as an important factor to confound the risk association of T2D with vitamin D because patients with T2D often coexist with central adiposity (Qiao and Nyamdorj, 2010; Vazquez *et al.*, 2007). Obese adults are more likely to be at high risk for vitamin D deficiency as body fat may sequester the fat-soluble vitamin (Holick *et al.*, 2011). Similar phenomena were also observed among children and adolescents who lived in different regions (e.g. 40.4 degree of N in Pittsburgh (Rajakumar *et al.*, 2011) and 33 degree of N in Atlanta (Dong *et al.*, 2010)) of the United States and Italy (Pacifico *et al.*, 2011), but not among children and adolescents living the Bangkok (13 degree of N) (Poomthavorn *et al.*, 2012) or adolescent girls in Beijing (40 degree of N), China (Foo *et al.*, 2009). Regardless of the obesity status, however, the mean levels of serum 25(OH) D for those children and adolescents in Bangkok were approximately 70 nmol/l; while for those girls in Beijing nearly 90% of them had serum 25(OH)D levels of <50 nmol/l, measured in winter time. This may suggest that obesity status may not be the issue as the majority of these subjects had either relatively high levels or relatively low levels of serum 25(OH) D. This question needs to be further explored. This may partially explain why in some studies adjustment for variables related to obesity (e.g. body mass index) the reversed risk association of T2D with serum 25(OH)D levels still persisted (Brock *et al.*, 2011; Scragg *et al.*, 2004), but in others were diminished (Grimnes *et al.*, 2010).

A number of interventional studies have been conducted to examine whether vitamin D supplement can reduce the risk of T2D, but the results are mixed. For instance, a double-blind, RCT was conducted among 314 older Caucasians without diabetes showed that in comparison to those in a placebo group, those in the experimental group with daily intake 700 IU of vitamin D3 and 500 mg of calcium for three years prevented increases in plasma glucose and insulin resistance among patients with impaired fasting glucose (Pittas *et al.*, 2007). Patients with T2D in another two RCTs also showed that patients who consumed a vitamin D3 fortified yogurt drink (1000 IU/day) with either 500 mg/day or less calcium in comparison to patients who consumed plain yogurt improved glycemic status, lipid profile and endothelial biomarkers (Nikooyeh *et al.*, 2011; Shab-Bidar *et al.*, 2011). However, the results from two large-scale RCTs, the RECORD and the WHI, did not show benefits of daily intake of vitamin D3 (800 IU/day alone or in combination with 1000 mg/day of calcium in the RECORD or 400 IU/day with 1000 mg/day of calcium in the WHI) in the prevention of the development of T2D (Avenell *et al.*, 2009; de Boer *et al.*, 2008). Several small RCT's results suggested that significant vitamin D supplementation (either in a single dose of 100,000 IU or 40,000 IU/week for 6 months) on patients with T2D did not improve their glycemic status (Jorde and Figenschau, 2009; Sugden *et al.*, 2008; Witham *et al.*, 2010), but it might improve systolic blood pressure and endothelial function in a short term (Sugden *et al.*, 2008; Weitzman *et al.*, 2010). However, no study has shown worsening pathology of T2D while taking vitamin D.

27.2.4 Vitamin D deficiency and gestational diabetes mellitus

The prevalence rates of GDM vary by region, but are not affected by latitude. A review done by the Vitamin D and Lifestyle Intervention for Gestational Diabetes Mellitus Prevention research group indicated that although the prevalence rates differed by regions in Europe (ranges 2.0-6.0%), the lower prevalence rates of GDM were in the Northern or Atlantic seaboard parts of Europe (<4%); while the higher prevalence rates (>6%) predominated in the South or Mediterranean seaboard regions (Buckley *et al.*, 2011). The results from the hyperglycemia and adverse pregnancy outcome study also showed that the frequency of GDM was not affected by the latitude, for example, those higher than 25% of GDM included those results from Bellflower, USA (33 degree of N) and Singapore (1 degree of N); while those results lower than 12% were from Barbados (13 degree of N) and Beersheba (31 degree of N) (Sacks *et al.*, 2012). However, whether the incidence of GDM differed by season seems unknown.

The results in regard to the relationship between vitamin D deficiency and GDM risk appear mixed with some of them in favor of it (Clifton-Bligh *et al.*, 2008; Soheilykhah *et al.*, 2010) and others not (Baker *et al.*, 2011; Farrant *et al.*, 2009). However, vitamin D deficiency (defined as <50 nmol/l) or insufficiency (<75-80 nmol/l) seems very prevalent in pregnant women (Dror, 2011; Hamilton *et al.*, 2011; Karim *et al.*, 2010; Teale and Cunningham, 2010). The levels of serum 25(OH) D have been observed to be inversely associated with levels of HbA_{1c} among women with GDM and this relationship seemed not to be affected by other known risk factors (Lau *et al.*, 2011). In addition, vitamin D deficiency has been linked to adverse outcomes of pregnancy, such as preeclampsia (Baker *et al.*, 2011; Robinson *et al.*, 2010) and small-for-gestational-age babies (Leffelaar *et al.*, 2010), but this is beyond the scope of our focus here and will be given more detail discussion in other section.

27.3 Vitamin D, bone size and diabetes mellitus

27.3.1 Bone size and diabetes mellitus

Although VDR is present in most tissues and cells in the body, suggesting that vitamin D may have been involved in a wide range of biological actions, its role on calcium/phosphate homeostasis to maintain bone health is considered as the primary and classic function. Interestingly, the relationship between bone size and diabetic risk has long been noticed. For example, the standing height, leg length, the ratio of leg length/height, and femur bone length are all observed to have a negative association with the risk of T2D, GDM, and glucose intolerance (Asao *et al.*, 2006; Brown *et al.*, 1991; Liu *et al.*, 2009; Njolstad *et al.*, 1998; Sayeed *et al.*, 1997; Weitzman *et al.*, 2010). However, in one study this negative relationship was only observed in white but not in black individuals (Weitzman *et al.*, 2010). Bone size and its risk for T1D appear not as sound as that for T2D because most incident T1D patients are still growing children and adolescents (Patterson *et al.*, 2009). Among T1D adults, however, it has been found that short stature is associated with microvascular complications (Waden *et al.*, 2009). One explanation for this observed relationship

is that these components of stature are indicators of factors acting during early childhood that affect the risk of developing diabetes in later life.

The new findings in bone metabolism suggest that bone is a critical endocrine organ and the molecular products from bone metabolism are involved in the global regulation of energy metabolism (Clemens and Karsenty, 2011). Osteocalcin, an osteoblast-specific protein during bone remodeling, is identified as a common link between bone and glucose metabolism in animal studies (Ferron *et al.*, 2010; Lee *et al.*, 2007). It was found in mice that osteocalcin can not only cause beta cells in the pancreas to release more insulin, but simultaneously, it can also direct fat cells to release more adiponectin, which increases sensitivity to insulin. The results from human studies, however, are mixed and depend on the type of diabetes and the stage. For instance, the low levels of osteocalcin at the baseline among normal males were observed to be associated with an increased risk for incident T2D in a nested case-control study (Ngarmukos *et al.*, 2011). Several studies have observed lower levels of osteocalcin in T1D patients with complications (Pasaoglu *et al.*, 1995) and male T2D patients (Kanazawa *et al.*, 2010). While for pregnant women, the levels of osteocalcin were higher in women with GDM than those without and it was suggested that osteocalcin may enhance insulin secretion in insulin resistance status (Winhofer *et al.*, 2010).

27.3.2 Serum 25(OH)D levels, femur bone size and type 2 diabetes mellitus

It would be too simple to consider that the bone size in the observed risk relationship with diabetes was just a proxy indicator of nutritional factors on early childhood development based on the emerging evidence. Osteocalcin may be the key to explain the observed relationship between bone size and the risk for diabetes, but no study so far has been done to examine the relationship between osteocalcin and bone size.

However, using the data from the national health and nutrition examination survey, we explored the relationship between serum 25(OH) D levels, femur bone size and T2D and found that the serum levels of 25(OH) D and all variables related to diabetes mellitus were negatively related to the gender-specific quintiles of femur bone lengths (Table 27.1) after adjustment for age, ethnicity, smoking, family history of diabetes, family income and waist circumference (Liu *et al.*, 2011).

When analysis was conducted among those without T2D, we also found that the odds ratios of impaired fasting glucose (between 5.6 mmol/l and 6.9 mmol/l; Shields, 2005) for quintiles 2-5 of femur bone lengths in comparison to the reference (quintile 1) were 0.94 (0.68, 1.31), 0.82 (0.59, 1.13), 0.76 (0.55, 1.05), and 0.63 (0.45, 0.86), respectively (P-value for trends <0.001).

VDR gene polymorphisms are found to have a link to adult height (Xiong *et al.*, 2005). It is believed that adequate intake of vitamin D will help not only children to reach their genetically programmed height and peak bone density but also will help adults to prevent osteoporosis (Holick, 2004). To maintain bone health, theoretically, taller adults may need more vitamin D than their shorter counterparts due to their longer bones that requiring more calcium. Since VDR is also found in beta cells, the active role of vitamin D in the functional regulation of the

Table 27.1. Adjusted[1] mean levels of vitamin D and other variables related to diabetes by quintile of femur bone length, the National Health and Nutrition Examination Survey (2001-2002,2003-2004) (Liu *et al.*, 2011: reproduced with permission).

	Femur length quintiles					*P*-value for trends
	Q1	Q2	Q3	Q4	Q5	
Males						
Range of femur length (cm)	~31.0	~39.4	~41.2	~43.0	45.0-53.4	
Serum 25-OH-vitamin D (ng/ml, mean)	23.7	24.2	24.6	25.0	25.4	*P*<0.05
Fasting plasma glucose (mg/dl, mean)	110.7	108.6	106.5	104.4	102.3	*P*<0.001
Fasting insulin (uu/ml, mean)	11.2	10.8	10.3	9.9	9.4	*P*<0.05
Glycohemoglobin (%, mean)	5.67	5.61	5.56	5.51	5.45	*P*<0.001
Diabetes (%)	11.9	10.0	8.4	7.0	5.8	*P*<0.001
Females						
Range of femur length (cm)	~26.4	~35.8	~37.7	~39.4	41.1-50.9	
Serum 25-OH-vitamin D (ng/ml, mean)	22.6	23.2	23.8	24.4	25.0	*P*<0.01
Fasting plasma glucose (mg/dl, mean)	102.7	101.2	99.8	98.3	96.8	*P*<0.001
Fasting insulin (uu/ml, mean)	10.4	9.9	9.4	8.9	8.4	*P*<0.001
Glycohemoglobin (%, mean)	5.61	5.55	5.49	5.44	5.38	*P*<0.001
Diabetes (%)	8.3	6.9	5.8	4.9	4.1	*P*<0.05

[1] Adjusted for age, current cigarette smoking, ethnicity, blood relatives with diabetes, family annual income and waist circumference.

endocrine pancreas is confirmed (Lee *et al.*, 1994). Therefore, if there is no restriction of the intake of vitamin D, the serum level of 25(OH) D in adults should reflect an ideal level to meet the physiological needs for both bone calcium homeostasis and glucose metabolism. However, in reality, the serum level of 25(OH) D may be lower than the ideal level in many adults because of inadequate intake of vitamin D levels from either exposure to sunlight or dietary sources (Hypponen and Power, 2007; Zadshir *et al.*, 2005).

It is unclear how bone size in adults and vitamin D levels influence diabetic risk development. However, VDR is also expressed in osteoblasts and regulates the expression of several genes in this cell, including osteocalcin (Lee *et al.*, 2000). Although no data has showed how serum levels of osteocalcin are related to adults' stature, the level of serum osteocalcin is recommended as one of the indicators in treatment monitoring of osteoporosis (Lindsay and Cosman, 2008). In normal children, the serum levels of osteocalcin increase as they grow and the peak value of it occurs when they reach puberty (Cioffi *et al.*, 1997; Kanzaki *et al.*, 1992). The observed negative diabetic risk associations with bone size in adults may be due to the capability of producing osteocalcin which is affected by vitamin D. Poor nutrition, including inadequate intake of vitamin

D in early childhood, affects children's growth, which may not only let the children have short bone size or short stature, but may also influence their glucose metabolic pathway. Those with serious deficiency of vitamin D may develop diabetes at an earlier age as shown by those Finnish children in whom those with suspected rickets during their first year of life had a 3-times higher risk of developing T1D later on (Hypponen *et al.*, 2001). However, this novel hypothesis needs to be further studied.

27.4 Conclusions

Emerging evidence suggests that vitamin D and diabetes mellitus are linked by biological associations. However, no clear evidence indicates a causal relationship between vitamin D deficiency and diabetes mellitus. In particular, the results from vitamin D supplementation studies are mixed and inconsistent, although no study has shown worsening of the pathology. Most previous studies on vitamin D and diabetes were orientated from the direction of its 'non-classic' effect of vitamin D, but the emerging evidence suggests that its 'classic' effect, on bone metabolism and via bone mediation, a newly recognized endocrine organ likely involved in energy metabolism and linked to the development of diabetes, may inform the direction of future research.

Acknowledgement

Thanks to Dr. Brian Jeynes for his helping in editing this manuscript.

References

Aanstoot, H.J., Anderson, B.J., Daneman, D., Danne, T., Donaghue, K., Kaufman, F., Rea, R.R. and Uchigata, Y., 2007. The global burden of youth diabetes: perspectives and potential. Pediatric Diabetes 8 Suppl 8, 1-44.

Abiru, N., Kawasaki, E. and Eguch, K., 2002. Current knowledge of Japanese type 1 diabetic syndrome. Diabetes/Metabolism Research and Reviews 18, 357-366.

Adams, J.S. and Hewison, M., 2010. Update in vitamin D. Journal of Clinical Endocrinology and Metabolism 95, 471-478.

Anderson, J.L., May, H.T., Horne, B.D., Bair, T.L., Hall, N.L., Carlquist, J.F., Lappe, D.L. and Muhlestein, J.B., 2010. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. American Journal of Cardiology 106, 963-968.

Anonymous, 1988. Geographic patterns of childhood insulin-dependent diabetes mellitus. Diabetes Epidemiology Research International Group. Diabetes 37, 1113-1119.

Anonymous, 2000. Variation and trends in incidence of childhood diabetes in Europe. EURODIAB ACE Study Group. Lancet 355, 873-876.

Asao, K., Kao, W.H., Baptiste-Roberts, K., Bandeen-Roche, K., Erlinger, T.P. and Brancati, F.L., 2006. Short stature and the risk of adiposity, insulin resistance, and type 2 diabetes in middle age: the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. Diabetes Care 29, 1632-1637.

Avenell, A., Cook, J.A., MacLennan, G.S. and McPherson, G.C., 2009. Vitamin D supplementation and type 2 diabetes: a substudy of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). Age and Ageing 38, 606-609.

Baker, A.M., Haeri, S., Camargo Jr., C.A., Stuebe, A.M. and Boggess, K.A., 2011. First-trimester maternal vitamin D status and risk for gestational diabetes (GDM) a nested case-control study. Diabetes/Metabolism Research and Reviews 28, 164-168.

Bellamy, L., Casas, J.P., Hingorani, A.D. and Williams, D., 2009. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet 373, 1773-1779.

Ben-Haroush, A., Yogev, Y. and Hod, M., 2004. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabetic Medicine 21, 103-113.

Bikle, D., 2009. Nonclassic actions of vitamin D. Journal of Clinical Endocrinology and Metabolism 94, 26-34.

Borkar, V.V., Devidayal, Verma, S. and Bhalla, A.K., 2010. Low levels of vitamin D in North Indian children with newly diagnosed type 1 diabetes. Pediatric Diabetes 11, 345-350.

Brock, K.E., Huang, W.Y., Fraser, D.R., Ke, L., Tseng, M., Mason, R.S., Stolzenberg-Solomon, R.Z., Freedman, D.M., Ahn, J., Peters, U., McCarty, C., Hollis, B.W., Ziegler, R.G., Purdue, M.P. and Graubard, B.I., 2011. Diabetes prevalence is associated with serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in US middle-aged Caucasian men and women: a cross-sectional analysis within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. British Journal of Nutrition 106, 339-344.

Brown, D.C., Byrne, C.D., Clark, P.M., Cox, B.D., Day, N.E., Hales, C.N., Shackleton, J.R., Wang, T.W. and Williams, D.R., 1991. Height and glucose tolerance in adult subjects. Diabetologia 34, 531-533.

Buckley, B.S., Harreiter, J., Damm, P., Corcoy, R., Chico, A., Simmons, D., Vellinga, A. and Dunne, F., 2011. Gestational diabetes mellitus in Europe: prevalence, current screening practice and barriers to screening. Diabetic Medicine 29, 844-854.

Cheng, Q., Li, Y.C., Boucher, B.J. and Leung, P.S., 2011. A novel role for vitamin D: modulation of expression and function of the local renin-angiotensin system in mouse pancreatic islets. Diabetologia 54, 2077-2081.

Cioffi, M., Molinari, A.M., Gazzerro, P., Di Finizio, B., Fratta, M., Deufemia, A. and Puca, G.A., 1997. Serum osteocalcin in 1634 healthy children. Clinical Chemistry 43, 543-545.

Clemens, T.L. and Karsenty, G., 2011. The osteoblast: an insulin target cell controlling glucose homeostasis. Journal of Bone and Mineral Research 26, 677-680.

Clifton-Bligh, R.J., McElduff, P. and McElduff, A., 2008. Maternal vitamin D deficiency, ethnicity and gestational diabetes. Diabetic Medicine 25, 678-684.

Concannon, P., Rich, S.S. and Nepom, G.T., 2009. Genetics of type 1A diabetes. New England Journal of Medicine 360, 1646-1654.

Cooper, J.D., Smyth, D.J., Walker, N.M., Stevens, H., Burren, O.S., Wallace, C., Greissl, C., Ramos-Lopez, E., Hypponen, E., Dunger, D.B., Spector, T.D., Ouwehand, W.H., Wang, T.J., Badenhoop, K. and Todd, J.A., 2011. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. Diabetes 60, 1624-1631.

Danaei, G., Finucane, M.M., Lu, Y., Singh, G.M., Cowan, M.J., Paciorek, C.J., Lin, J.K., Farzadfar, F., Khang, Y.H., Stevens, G.A., Rao, M., Ali, M.K., Riley, L.M., Robinson, C.A. and Ezzati, M., 2011. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 378, 31-40.

Daneman, D., 2006. Type 1 diabetes. Lancet 367, 847-858.

De Boer, I.H., Tinker, L.F., Connelly, S., Curb, J.D., Howard, B.V., Kestenbaum, B., Larson, J.C., Manson, J.E., Margolis, K.L., Siscovick, D.S. and Weiss, N.S., 2008. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 31, 701-707.

Devendra, D., Liu, E. and Eisenbarth, G.S., 2004. Type 1 diabetes: recent developments. BMJ 328, 750-754.

Dong, Y., Pollock, N., Stallmann-Jorgensen, I.S., Gutin, B., Lan, L., Chen, T.C., Keeton, D., Petty, K., Holick, M.F. and Zhu, H., 2010. Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. Pediatrics 125, 1104-1111.

Driver, J.P., Lamont, D.J., Gysemans, C., Mathieu, C. and Serreze, D.V., 2011. Calcium insufficiency accelerates type 1 diabetes in vitamin D receptor-deficient nonobese diabetic (NOD) mice. Endocrinology 152, 4620-4629.

Dror, D.K., 2011. Vitamin D status during pregnancy: maternal, fetal, and postnatal outcomes. Current Opinion in Obstetrics and Gynecology 23, 422-426.

Farrant, H.J., Krishnaveni, G.V., Hill, J.C., Boucher, B.J., Fisher, D.J., Noonan, K., Osmond, C., Veena, S.R. and Fall, C.H., 2009. Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. European Journal of Clinical Nutrition 63, 646-652.

Ferron, M., Wei, J., Yoshizawa, T., Del Fattore, A., DePinho, R.A., Teti, A., Ducy, P. and Karsenty, G., 2010. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 142, 296-308.

Fleegler, F., Rogers, K.D., Drash, A., Travis, L.B. and Rosenbloom, A.L., 1978. The season and diabetes. Pediatrics 61, 325.

Foo, L.H., Zhang, Q., Zhu, K., Ma, G., Trube, A., Greenfield, H. and Fraser, D.R., 2009. Relationship between vitamin D status, body composition and physical exercise of adolescent girls in Beijing. Osteoporosis International 20, 417-425.

Fujita, T., 2000. Calcium paradox: consequences of calcium deficiency manifested by a wide variety of diseases. Journal of Bone and Mineral Metabolism 18, 234-236.

Garland, C.F., Comstock, G.W., Garland, F.C., Helsing, K.J., Shaw, E.K. and Gorham, E.D., 1989. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet 2, 1176-1178.

Garland, F.C., Garland, C.F., Gorham, E.D. and Young, J.F., 1990. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. Preventive Medicine 19, 614-622.

Giulietti, A., Gysemans, C., Stoffels, K., van Etten, E., Decallonne, B., Overbergh, L., Bouillon, R. and Mathieu, C., 2004. Vitamin D deficiency in early life accelerates Type 1 diabetes in non-obese diabetic mice. Diabetologia 47, 451-462.

Gogas Yavuz, D., Keskin, L., Kiyici, S., Sert, M., Yazici, D., Sahin, I., Yuksel, M., Deyneli, O., Aydin, H., Tuncel, E. and Akalin, S., 2011. Vitamin D receptor gene BsmI, FokI, ApaI, TaqI polymorphisms and bone mineral density in a group of Turkish type 1 diabetic patients. Acta Diabetologica 48, 329-336.

Grimnes, G., Emaus, N., Joakimsen, R.M., Figenschau, Y., Jenssen, T., Njolstad, I., Schirmer, H. and Jorde, R., 2010. Baseline serum 25-hydroxyvitamin D concentrations in the Tromso Study 1994-95 and risk of developing type 2 diabetes mellitus during 11 years of follow-up. Diabetic Medicine 27, 1107-1115.

Guo, S.W., Magnuson, V.L., Schiller, J.J., Wang, X., Wu, Y. and Ghosh, S., 2006. Meta-analysis of vitamin D receptor polymorphisms and type 1 diabetes: a HuGE review of genetic association studies. American Journal of Epidemiology 164, 711-724.

Gysemans, C., van Etten, E., Overbergh, L., Giulietti, A., Eelen, G., Waer, M., Verstuyf, A., Bouillon, R. and Mathieu, C., 2008. Unaltered diabetes presentation in NOD mice lacking the vitamin D receptor. Diabetes 57, 269-275.

Hamilton, S.A., McNeil, R., Hollis, B.W., Davis, D.J., Winkler, J., Cook, C., Warner, G., Bivens, B., McShane, P. and Wagner, C.L., 2011. Profound Vitamin D Deficiency in a Diverse Group of Women during Pregnancy Living in a Sun-Rich Environment at Latitude 32 degrees N. International Journal of Endocrinology 2010, 917428.

Hawa, M.I., Valorani, M.G., Buckley, L.R., Beales, P.E., Afeltra, A., Cacciapaglia, F., Leslie, R.D. and Pozzilli, P., 2004. Lack of effect of vitamin D administration during pregnancy and early life on diabetes incidence in the non-obese diabetic mouse. Hormone and Metabolic Research 36, 620-624.

Holick, M.F., 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. American Journal of Clinical Nutrition 80, 1678S-1688S.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Holick, M.F., 2009. Vitamin D status: measurement, interpretation, and clinical application. Annals of Epidemiology 19, 73-78.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M., 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism 96, 1911-1930.

Hossain, P., Kawar, B. and El Nahas, M., 2007. Obesity and diabetes in the developing world – a growing challenge. New England Journal of Medicine 356, 213-215.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R. and Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358, 1500-1503.

Hypponen, E. and Power, C., 2007. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. American Journal of Clinical Nutrition 85, 860-868.

IDF, 2009. Diabetes in the Young: a Global Perspective, IDF Diabetes Atlas. International Diabetes Federation.

Ishii, H., Suzuki, H., Baba, T., Nakamura, K. and Watanabe, T., 2001. Seasonal variation of glycemic control in type 2 diabetic patients. Diabetes Care 24, 1503.

Jorde, R. and Figenschau, Y., 2009. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. European Journal of Nutrition 48, 349-354.

Kahn, R. and Anderson, J.E., 2009. Improving diabetes care: the model for health care reform. Diabetes Care 32, 1115-1118.

Kanazawa, I., Yamaguchi, T., Yamauchi, M., Yamamoto, M., Kurioka, S., Yano, S. and Sugimoto, T., 2010. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. Osteoporosis International 22, 187-194.

Kanzaki, S., Hosoda, K., Moriwake, T., Tanaka, H., Kubo, T., Inoue, M., Higuchi, J., Yamaji, T. and Seino, Y., 1992. Serum propeptide and intact molecular osteocalcin in normal children and children with growth hormone (GH) deficiency: a potential marker of bone growth and response to GH therapy. Journal of Clinical Endocrinology and Metabolism 75, 1104-1109.

Karim, S.A., Nusrat, U. and Aziz, S., 2010. Vitamin D deficiency in pregnant women and their newborns as seen at a tertiary-care center in Karachi, Pakistan. International Journal of Gynaecology and Obstetrics 112, 59-62.

Kirii, K., Mizoue, T., Iso, H., Takahashi, Y., Kato, M., Inoue, M., Noda, M. and Tsugane, S., 2009. Calcium, vitamin D and dairy intake in relation to type 2 diabetes risk in a Japanese cohort. Diabetologia 52, 2542-2550.

Knekt, P., Laaksonen, M., Mattila, C., Harkanen, T., Marniemi, J., Heliovaara, M., Rissanen, H., Montonen, J. and Reunanen, A., 2008. Serum vitamin D and subsequent occurrence of type 2 diabetes. Epidemiology 19, 666-671.

Lain, K.Y. and Catalano, P.M., 2007. Metabolic changes in pregnancy. Clinical Obstetrics and Gynecology 50, 938-948.

Lau, S.L., Gunton, J.E., Athayde, N.P., Byth, K. and Cheung, N.W., 2011. Serum 25-hydroxyvitamin D and glycated haemoglobin levels in women with gestational diabetes mellitus. Medical Journal of Australia 194, 334-337.

Lee, A.J., Hodges, S. and Eastell, R., 2000. Measurement of osteocalcin. Annals of Clinical Biochemistry 37 (Pt 4), 432-446.

Lee, N.K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J.D., Confavreux, C., Dacquin, R., Mee, P.J., McKee, M.D., Jung, D.Y., Zhang, Z., Kim, J.K., Mauvais-Jarvis, F., Ducy, P. and Karsenty, G., 2007. Endocrine regulation of energy metabolism by the skeleton. Cell 130, 456-469.

Lee, S., Clark, S.A., Gill, R.K. and Christakos, S., 1994. 1,25-Dihydroxyvitamin D3 and pancreatic beta-cell function: vitamin D receptors, gene expression, and insulin secretion. Endocrinology 134, 1602-1610.

Leffelaar, E.R., Vrijkotte, T.G. and van Eijsden, M., 2010. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. British Journal of Nutrition 104, 108-117.

Liang, W.W., 2007. Seasonal changes in preprandial glucose, A1C, and blood pressure in diabetic patients. Diabetes Care 30, 2501-2502.

Lindsay, R. and Cosman, F., 2008. Osteoporosis. In: A. Fauci, E. Braunwald, D. Kasper, S. Hauser, D. Longo, J.L. Jameson and J. Loscalzo (eds.), Harrison's Principles of Internal Medicine. Mc Graw Hill, New York, pp. 2397-2408.

Littorin, B., Blom, P., Scholin, A., Arnqvist, H.J., Blohme, G., Bolinder, J., Ekbom-Schnell, A., Eriksson, J.W., Gudbjornsdottir, S., Nystrom, L., Ostman, J. and Sundkvist, G., 2006. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 49, 2847-2852.

Liu, J., Sempos, C., Donahue, R.P., Dorn, J., Trevisan, M. and Grundy, S.M., 2005. Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes. Diabetes Care 28, 1916-1921.

Liu, J., Tan, H. and Jeynes, B., 2009. Is femur length the key height component in risk prediction of type 2 diabetes among adults? Diabetes Care 32, 739-740.

Liu, J., Tan, H. and Jeynes, B., 2011. Serum 25OH vitamin D level, femur length, and risk of type 2 diabetes among adults. Applied Physiology, Nutrition, and Metabolism 36, 264-270.

Marjamaki, L., Niinisto, S., Kenward, M.G., Uusitalo, L., Uusitalo, U., Ovaskainen, M.L., Kronberg-Kippila, C., Simell, O., Veijola, R., Ilonen, J., Knip, M. and Virtanen, S.M., 2010. Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. Diabetologia 53, 1599-1607.

Mathieu, C., Waer, M., Laureys, J., Rutgeerts, O. and Bouillon, R., 1994. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. Diabetologia 37, 552-558.

Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhardt, P., Dawson-Hughes, B., Eisman, J.A., El-Hajj Fuleihan, G., Josse, R.G., Lips, P. and Morales-Torres, J., 2009. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International 20, 1807-1820.

Mohr, S.B., Garland, C.F., Gorham, E.D. and Garland, F.C., 2008. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. Diabetologia 51, 1391-1398.

Moltchanova, E.V., Schreier, N., Lammi, N. and Karvonen, M., 2009. Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwide. Diabetic Medicine 26, 673-678.

Nagpal, S., Na, S. and Rathnachalam, R., 2005. Noncalcemic actions of vitamin D receptor ligands. Endocrine Reviews 26, 662-687.

Ngarmukos, C., Chailurkit, L.O., Chanprasertyothin, S., Hengprasith, B., Sritara, P. and Ongphiphadhanakul, B., 2011. A reduced serum level of total osteocalcin in men predicts development of diabetes in a long-term follow-up cohort. Clinical Endocrinology 77, 42-46.

Nikooyeh, B., Neyestani, T.R., Farvid, M., Alavi-Majd, H., Houshiarrad, A., Kalayi, A., Shariatzadeh, N., Gharavi, A., Heravifard, S., Tayebinejad, N., Salekzamani, S. and Zahedirad, M., 2011. Daily consumption of vitamin D- or vitamin D + calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. American Journal of Clinical Nutrition 93, 764-771.

Njolstad, I., Arnesen, E. and Lund-Larsen, P.G., 1998. Sex differences in risk factors for clinical diabetes mellitus in a general population: a 12-year follow-up of the Finnmark Study. American Journal of Epidemiology 147, 49-58.

Osei, K., 2010. 25-OH vitamin D: is it the universal panacea for metabolic syndrome and type 2 diabetes? Journal of Clinical Endocrinology and Metabolism 95, 4220-4222.

Pacifico, L., Anania, C., Osborn, J.F., Ferraro, F., Bonci, E., Olivero, E. and Chiesa, C., 2011. Low 25(OH)D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. European Journal of Endocrinology 165, 603-611.

Pasaoglu, H., Kumandas, S. and Kelestimur, F., 1995. Serum osteocalcin levels in type I diabetes mellitus. Turkish Journal of Pediatrics 37, 323-329.

Patterson, C.C., Dahlquist, G.G., Gyurus, E., Green, A. and Soltesz, G., 2009. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 373, 2027-2033.

Pettifor, J.M., Moodley, G.P., Hough, F.S., Koch, H., Chen, T., Lu, Z. and Holick, M.F., 1996. The effect of season and latitude on *in vitro* vitamin D formation by sunlight in South Africa. South African Medical Journal 86, 1270-1272.

Pittas, A.G., Chung, M., Trikalinos, T., Mitri, J., Brendel, M., Patel, K., Lichtenstein, A.H., Lau, J. and Balk, E.M., 2010. Systematic review: Vitamin D and cardiometabolic outcomes. Annals of Internal Medicine 152, 307-314.

Pittas, A.G., Dawson-Hughes, B., Li, T., Van Dam, R.M., Willett, W.C., Manson, J.E. and Hu, F.B., 2006. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 29, 650-656.

Pittas, A.G., Harris, S.S., Stark, P.C. and Dawson-Hughes, B., 2007. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 30, 980-986.

Ponsonby, A.L., Pezic, A., Ellis, J., Morley, R., Cameron, F., Carlin, J. and Dwyer, T., 2008. Variation in associations between allelic variants of the vitamin D receptor gene and onset of type 1 diabetes mellitus by ambient winter ultraviolet radiation levels: a meta-regression analysis. American Journal of Epidemiology 168, 358-365.

Poomthavorn, P., Saowan, S., Mahachoklertwattana, P., Chailurkit, L. and Khlairit, P., 2012. Vitamin D status and glucose homeostasis in obese children and adolescents living in the tropics. International Journal of Obesity (2005) 36, 491-495.

Pozzilli, P., Manfrini, S., Crino, A., Picardi, A., Leomanni, C., Cherubini, V., Valente, L., Khazrai, M. and Visalli, N., 2005. Low levels of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. Hormone and Metabolic Research 37, 680-683.

Qiao, Q. and Nyamdorj, R., 2010. Is the association of type II diabetes with waist circumference or waist-to-hip ratio stronger than that with body mass index? European Journal of Clinical Nutrition 64, 30-34.

Rajakumar, K., de las Heras, J., Chen, T.C., Lee, S., Holick, M.F. and Arslanian, S.A., 2011. Vitamin D status, adiposity, and lipids in black American and Caucasian children. Journal of Clinical Endocrinology and Metabolism 96, 1560-1567.

Reece, E.A., Leguizamon, G. and Wiznitzer, A., 2009. Gestational diabetes: the need for a common ground. Lancet 373, 1789-1797.

Risch, N., 1987. Assessing the role of HLA-linked and unlinked determinants of disease. American Journal of Human Genetics 40, 1-14.

Robinson, C.J., Alanis, M.C., Wagner, C.L., Hollis, B.W. and Johnson, D.D., 2010. Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. American Journal of Obstetrics and Gynecology 203, 366 e361-366.

Ross, A.C., Taylor, C.L., Yaktine, A.L. and Del Valle, H.B., 2011. Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press.

Sacks, D.A., Hadden, D.R., Maresh, M., Deerochanawong, C., Dyer, A.R., Metzger, B.E., Lowe, L.P., Coustan, D.R., Hod, M., Oats, J.J., Persson, B. and Trimble, E.R., 2012. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Diabetes Care 35, 526-528.

Sayeed, M.A., Hussain, M.Z., Banu, A., Rumi, M.A. and Azad Khan, A.K., 1997. Prevalence of diabetes in a suburban population of Bangladesh. Diabetes Research and Clinical Practice 34, 149-155.

Scragg, R., Holdaway, I., Singh, V., Metcalf, P., Baker, J. and Dryson, E., 1995. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. Diabetes Research and Clinical Practice 27, 181-188.

Scragg, R., Sowers, M. and Bell, C., 2004. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 27, 2813-2818.

Shab-Bidar, S., Neyestani, T.R., Djazayery, A., Eshraghian, M.R., Houshiarrad, A., Gharavi, A., Kalayi, A., Shariatzadeh, N., Zahedirad, M., Khalaji, N. and Haidari, H., 2011. Regular consumption of vitamin D-fortified yogurt drink (Doogh) improved endothelial biomarkers in subjects with type 2 diabetes: a randomized double-blind clinical trial. BMC Medicine 9, 125.

Shields, M., 2005. Measured Obesity Overweight Canadian children and adolescents. Nutrition: Findings from the Canadian Community Health Survey (Issue no. 1). Statistics Canada, Ottawa.

Simpson, M., Brady, H., Yin, X., Seifert, J., Barriga, K., Hoffman, M., Bugawan, T., Baron, A.E., Sokol, R.J., Eisenbarth, G., Erlich, H., Rewers, M. and Norris, J.M., 2011. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia 54, 2779-2788.

Sloka, S., Grant, M. and Newhook, L.A., 2010. The geospatial relation between UV solar radiation and type 1 diabetes in Newfoundland. Acta Diabetologica 47, 73-78.

Soheilykhah, S., Mojibian, M., Rashidi, M., Rahimi-Saghand, S. and Jafari, F., 2010. Maternal vitamin D status in gestational diabetes mellitus. Nutrition in Clinical Practice 25, 524-527.

Sorensen, I.M., Joner, G., Jenum, P.A., Eskild, A., Torjesen, P.A. and Stene, L.C., 2011. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. Diabetes 61, 175-178.

Staples, J.A., Ponsonby, A.L., Lim, L.L. and McMichael, A.J., 2003. Ecologic analysis of some immune-related disorders, including type 1 diabetes, in Australia: latitude, regional ultraviolet radiation, and disease prevalence. Environmental Health Perspectives 111, 518-523.

Stene, L.C. and Joner, G., 2003. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. American Journal of Clinical Nutrition 78, 1128-1134.

Stene, L.C., Ulriksen, J., Magnus, P. and Joner, G., 2000. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 43, 1093-1098.

Sugden, J.A., Davies, J.I., Witham, M.D., Morris, A.D. and Struthers, A.D., 2008. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabetic Medicine 25, 320-325.

Svoren, B.M., Volkening, L.K., Wood, J.R. and Laffel, L.M., 2009. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. Journal of Pediatrics 154, 132-134.

Teale, G.R. and Cunningham, C.E., 2010. Vitamin D deficiency is common among pregnant women in rural Victoria. Australian and New Zealand Journal of Obstetrics and Gynaecology 50, 259-261.

Van der Mei, I.A., Ponsonby, A.L., Engelsen, O., Pasco, J.A., McGrath, J.J., Eyles, D.W., Blizzard, L., Dwyer, T., Lucas, R. and Jones, G., 2007. The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. Environmental Health Perspectives 115, 1132-1139.

Vazquez, G., Duval, S., Jacobs Jr., D.R. and Silventoinen, K., 2007. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. Epidemiologic Reviews 29, 115-128.

Waden, J., Forsblom, C., Thorn, L.M., Saraheimo, M., Rosengard-Barlund, M., Heikkila, O., Hietala, K., Ong, K., Wareham, N. and Groop, P.H., 2009. Adult stature and diabetes complications in patients with type 1 diabetes: the FinnDiane Study and the diabetes control and complications trial. Diabetes 58, 1914-1920.

Weets, I., Truyen, I., Verschraegen, I., Van der Auwera, B., De Schepper, J., Dorchy, H., Lebrethon, M.C., Van Gaal, L., Van Rooy, P., Pipeleers, D.G. and Gorus, F.K., 2006. Sex- and season-dependent differences in C-peptide levels at diagnosis of immune-mediated type 1 diabetes. Diabetologia 49, 1158-1162.

Weitzman, S., Wang, C.H., Pankow, J.S., Schmidt, M.I. and Brancati, F.L., 2010. Are measures of height and leg length related to incident diabetes mellitus? The ARIC (Atherosclerosis Risk in Communities) study. Acta Diabetologica 47, 237-242.

WHO, 2011a. Diabetes. World Health Organization, Geneva, Switzerland.

WHO, 2011b. Global Status Report on noncommunicable diseases 2010. World Health Organization, geneva, Switzerland.

Winhofer, Y., Handisurya, A., Tura, A., Bittighofer, C., Klein, K., Schneider, B., Bieglmayer, C., Wagner, O.F., Pacini, G., Luger, A. and Kautzky-Willer, A., 2010. Osteocalcin is related to enhanced insulin secretion in gestational diabetes mellitus. Diabetes Care 33, 139-143.

Witham, M.D., Dove, F.J., Dryburgh, M., Sugden, J.A., Morris, A.D. and Struthers, A.D., 2010. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 53, 2112-2119.

Wolden-Kirk, H., Overbergh, L., Christesen, H.T., Brusgaard, K. and Mathieu, C., 2011. Vitamin D and diabetes: its importance for beta cell and immune function. Molecular and Cellular Endocrinology 347, 106-120.

Xiong, D.H., Xu, F.H., Liu, P.Y., Shen, H., Long, J.R., Elze, L., Recker, R.R. and Deng, H.W., 2005. Vitamin D receptor gene polymorphisms are linked to and associated with adult height. Journal of Medical Genetics 42, 228-234.

Zadshir, A., Tareen, N., Pan, D., Norris, K. and Martins, D., 2005. The prevalence of hypovitaminosis D among US adults: data from the NHANES III. Ethnicity & Disease 15, S5-97-101.

Zella, J.B., McCary, L.C. and DeLuca, H.F., 2003. Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. Archives of Biochemistry and Biophysics 417, 77-80.

Zhang, F., Dong, L., Zhang, C.P., Li, B., Wen, J., Gao, W., Sun, S., Lv, F., Tian, H., Tuomilehto, J., Qi, L., Zhang, C.L., Yu, Z., Yang, X. and Hu, G., 2011. Increasing prevalence of gestational diabetes mellitus in Chinese women from 1999 to 2008. Diabetic Medicine 28, 652-657.

Zimmet, P., Alberti, K.G. and Shaw, J., 2001. Global and societal implications of the diabetes epidemic. Nature 414, 782-787.

Key facts

- Cardiovascular disease (CVD) is the number one cause of morbidity and mortality in adults in the Western World and is rapidly emerging as a major health concern in developing nations.
- Vitamin D receptors now are known to be found in heart and vascular tissue as well as bone, connective tissue, and many other tissues and organs, where they regulate the expression of multiple genes.
- Vitamin D deficiency is common among subjects at risk for and patients with established CVDs.
- Vitamin D deficiency now has been associated in multiple populations with cardiovascular risk factors and CVDs, including hypertension, obesity, diabetes, hyperlipidemia, coronary artery disease, myocardial infarction, heart failure, renal failure, and cardiovascular death.
- Observational studies to date supporting a role for vitamin D replacement in reducing cardiovascular risk are promising but limited, and randomized trials are needed.

Summary points

- CVD is a leading cause of death and disability worldwide. The discovery of new risk factors and preventive measures for CVD is of major importance.
- Vitamin D deficiency is common among subjects at risk for and patients with established CVDs. In a large healthcare population database, an initial measured 25(OH)D level was ≤30 ng/ml in 64% and, importantly, ≤15 ng/ml (deficient) in 17%.
- Chronic vitamin D deficiency leads to secondary hyperparathyroidism, which acts to increase insulin resistance and pancreatic β-cell dysfunction, activate the renin-angiotensin system, increasing blood pressure, and stimulate vascular inflammation, augmenting atherogenesis.
- A 'U' shaped association between vitamin D and health outcomes has been suggested, but both the lower and upper optimal boundaries for 25(OH)D remain controversial. The US Institute of Medicine report suggests that levels >20 ng/ml are adequate with no evidence for greater benefit of >30 ng/ml and possible harm at >50 ng/ml.
- Studies assessing a role for vitamin D replacement to reduce cardiovascular risk are limited, with some but not all suggesting benefit.
- Given inconclusive evidence, randomized clinical trials are needed to demonstrate that supplementation can play a major role in reducing cardiovascular risk factors and preventing or treating CVD. One trial, VITAL, in 20,000 subjects is underway. However, given design limitations of VITAL, other trials may be required.
- Pending additional evidence, either an empiric approach to lifestyle and Vitamin D supplementation or selected screening and treating to target may be considered.

28. Vitamin D and cardiovascular risk: hype or new hope?

J.L. Anderson and J.B. Muhlestein
Intermountain Medical Center, Cardiovascular Department, 5121 S. Cottonwood Street, Murray, UT 84107-5701, USA; jeffrey.anderson@imail.org

Abstract

Cardiovascular disease (CVD) is a leading cause of death and disability worldwide. The discovery of new risk factors and preventive measures for CVD is of major importance. Vitamin D receptors now are known to exist in heart and vascular tissue as well as bone, connective tissue, and many other tissues and organs, where they regulate the expression of multiple genes. Low or deficient levels of vitamin D are common among subjects at risk for and patients with established cardiovascular diseases. Importantly, vitamin D deficiency now has been associated in multiple populations with cardiovascular risk factors and cardiovascular diseases, including hypertension, obesity, diabetes, hyperlipidemia, coronary artery disease, myocardial infarction, heart failure, renal failure, and cardiovascular death. Multiple mechanisms relating vitamin D deficiency and CVD have been proposed, including secondary hyperparathyroidism. Studies to date supporting a role for vitamin D replacement to reduce CV risk are promising but limited and mostly observational, and not all recent evidence has been supportive of either CVD associations or benefits of vitamin D supplementation. Hence, randomized trials, such as VITAL, now underway, will be needed to firmly establish in whom and how to supplement vitamin D to improve CV health and reduce risk. A 'U' shaped association for vitamin D and health outcomes has been suggested, but both the lower and upper boundary of the optimal range for 25(OH)D levels for CV health remain controversial. The US Institute of Medicine report suggests a range 20-50 ng/ml as adequate and safe, but additional evidence is needed specific to CV health. Pending additional clinical trials evidence, both an empiric approach to lifestyle and supplementation or selected screening and treating to target may be considered. In conclusion, vitamin D deficiency has been shown to be associated with CV risk and disease, but more robust evidence is needed to conclusively demonstrate that vitamin D supplementation can play a major role in preventing or treating CVD.

Keywords: coronary artery disease, diabetes, heart failure, hypertension, mortality

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_28, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AFib	Atrial fibrillation
CAD	Coronary artery disease
CV	Cardiovascular
CVA	Stroke/cerebrovascular accident
CVD	Cardiovascular disease
eMR	Electronic medical records
HR	Hazard ratio
IOM	US Institute of Medicine
MI	Myocardial infarction
NHANES	National Health and Nutrition Examination Survey
PTH	Parathyroid hormone
RR	Relative risk
UVB	Ultraviolet B
VDR	Vitamin D receptor
VITAL	Vitamin D and omega-3 trial

28.1 Vitamin D: a brief overview

Beyond bone health, the role of vitamin D in general health and a broad spectrum of common disorders has been the focus of great interest in the past few years and of several excellent contemporary reviews (Holick, 2007; Lee *et al.*, 2008; McGreevy and Williams, 2011; Wallis *et al.*, 2008). Among these areas of interest is the role of vitamin D in CV health and disease (Lavie *et al.*, 2011; McGreevy and Williams, 2011; Wang *et al.*, 2008). CVD is the leading cause of morbidity and mortality in adults in the Western World and is rapidly emerging as a major entity in the developing world (Roger *et al.*, 2012; Yusuf *et al.*, 2001). Hence, a better understanding of underlying risk factors for CVD and new approaches to prevention are of great interest. Vitamin D is a vitamin whose functions simulate those of a hormone. It is derived from cholesterol; it requires 2-3 organs for activation (skin [except for dietary sources], liver, and kidney); VDR (principally in nuclei of target cells) are found in multiple cells and tissues, including cardiac myocytes and vascular tissue, and most organs. vitamin D works systemically on these multiple tissues/organs (e.g. bone/connective tissue, muscle, kidney, pancreas, parathyroid, brain, gonads, blood vessels, heart, etc.) where it modulates expression of a vast collection of genes (estimated at >200). Hence, variations in vitamin D availability have the potential to impact function, and deficiency to cause dysfunction, of multiple organ systems besides bone and connective tissue, including the CV system.

28.2 Metabolic forms

Vitamin D, synthesized in the skin from cholesterol in response to sunlight (UVB), is hydroxylated in the liver to 25(OH)D (McGreevy and Williams, 2011). 25(OH)D serves as the principal circulating and storage form of vitamin D and is generally accepted as the best measure of total body vitamin D stores. 25(OH)D is commonly measured in serum or plasma to assess vitamin D status. The kidney (primarily) and other organs further hydroxylate 25(OH)D to 1, $25(OH)_2D$, the active form of vitamin D. $1,25(OH)_2D$ is an important regulator of PTH secretion (Holick, 2007).

28.3 Selected non-skeletal functions

VDR activation in the intestine, bone, kidney, and parathyroid gland cells is well recognized to be essential to calcium absorption and the maintenance of calcium and phosphorus levels in blood (in association with PTH and calcitonin) and of bone mineral content. Increasingly, important functions of vitamin D outside of the skeletal system are being recognized and are summarized in other chapters; these sites include pancreas, breast, colon, prostate, and the immune system (e.g. lymphocytes) (Holick, 2007).

28.4 Defining vitamin D deficiency

An important prerequisite to identifying populations at risk is to accurately define vitamin D deficiency. Unfortunately, defining vitamin D deficiency evokes significant controversy (Dawson-Hughes *et al.*, 2010; Kennel *et al.*, 2010; McGreevy and Williams, 2011; Ross *et al.*, 2011). Commonly, 25(OH)D sufficiency has been defined as ≥30 ng/ml (≥75 nmol/l), i.e. with a normal range of 30-74 ng/ml, insufficiency (mild deficiency) as 20-29 ng/ml, moderate deficiency as 10-19 ng/ml, and severe deficiency as <10 ng/ml, and toxicity as >100-150 ng/ml. One school of thought has contended that exposure of humans in native, outdoor settings generally results in plasma 25(OH)D levels of 40-80 ng/ml. However, a recent (US) IOM report concluded that a serum 25(OH)D level of 20 ng/ml (50 nmol/l) is adequate to maintain bone and overall health, that concentrations above 30 ng/ml are 'not consistently associated with increased benefit', and that levels above 50 ng/ml (125 nmol/l) may be a cause for concern (Ross *et al.*, 2011). As will be seen, the choice of ≥20 versus ≥30 ng/ml as a definition for 25(OH)D sufficiency makes a major difference in the size of the population at risk and the target for supplemental treatment.

One approach to defining 25(OH)D deficiency is to note the concentration at which levels of PTH rise, as PTH is negatively regulated by 25(OH)D. PTH rises modestly as 25(OH)D levels decline from 30 to 20 ng/ml, then briskly at 25(OH)D levels ≤15 ng/ml (Zitterman, 2006).

28.5 Vitamin D deficiency: an international epidemic?

The major portion of circulating vitamin D is supplied by exposure to sunlight, with a minor contribution coming from common dietary sources. Several features of geography and modern lifestyle have converged to limit average exposure to sunlight and to contribute to a growing population trend toward vitamin D deficiency (Table 28.1). In addition, genetic factors may add to inter-individual variability in levels given the same dietary intake and sunlight exposure (Shirts *et al.*, 2012; Wang *et al.*, 2010).

In the setting of these and other contemporary lifestyle and geographic factors, an important percentage (i.e. 24-93%) of various population groups has been reported to be vitamin D deficient (Anderson *et al.*, 2010; Lee *et al.*, 2008; Tangpricha *et al.*, 2002; Wang *et al.*, 2008).

It should be noted that levels of <30 ng/ml have often been defined as deficient in many reports, whereas levels of <10-20 ng/ml may be more relevant to disease predisposition based on the recent IOM report (Ross *et al.*, 2011) and affect a smaller (though still important) segment of these populations. A Europe-wide survey reported a prevalence of severe vitamin D deficiency (<25 nmol/l=<10 ng/ml) in 2% to 30% of adults, but this increased to 75% or more in older, institutionalized persons (Lips, 2001). These very deficient levels are particularly prevalent in women in South Asia and the Middle East where cultural dress limits sun exposure and where extended periods of breastfeeding without vitamin D supplementation are common (Mithal *et al.*, 2009). In our experience in the United States, of 41,504 subjects in the Intermountain Healthcare eMR database with a measured vitamin D level, 64% had an initial level ≤30 ng/ml, and 17%, ≤15 ng/ml.

Table 28.1. Factors predisposing to vitamin D deficiency.

Advanced age
Hospitalized, institutionalized, homebound persons
Indoor lifestyle and sun avoidance
Sun protection (sunscreen, heavy clothing)
Darker skin and higher latitudes (changes in traditional racial/ethnic geographies)
Obesity, diabetes
Decreased fortified milk consumption
Smoking, air pollution
Kidney disease, liver disease, malabsorption syndromes

28.6 Vitamin D and disease associations

In recent years, a number of disease associations with vitamin D deficiency beyond osteoporosis and myalgias have been described. These include various cancers (e.g. colorectal, breast, prostate), chronic kidney disease, types 1 and 2 diabetes mellitus, hypertension, obesity, rheumatoid arthritis, multiple sclerosis, depression and cognitive impairment, and CV diseases (Holick, 2007; Lee *et al.*, 2008; McGreevy and Williams, 2011; Wallis *et al.*, 2008; Wang *et al.*, 2008). This chapter now will focus on potential links of vitamin D deficiency to CV risk and CVD.

28.7 Proposed mechanisms of cardiovascular risk

A leading explanation for a relationship between vitamin D deficiency and CVD is that chronic vitamin D deficiency leads to secondary hyperparathyroidism, which then can act through at least 3 pathogenic pathways to increase CV risk (Lee *et al.*, 2008; Wallis *et al.*, 2008): (1) increased insulin resistance and pancreatic β-cell dysfunction, predisposing to the metabolic syndrome and diabetes; (2) activation of the renin-angiotensin system, increasing blood pressure and leading to left ventricular hypertrophy (with subsequent myocyte apoptosis and cardiac fibrosis); and (3) stimulation of systemic and vascular inflammation, augmenting atherogenesis. These relationships are shown in Figure 28.1.

However, PTH elevation appears to explain only part the association of CV risk with vitamin D deficiency. In a large (9,369) study in a general healthcare population, we found that PTH

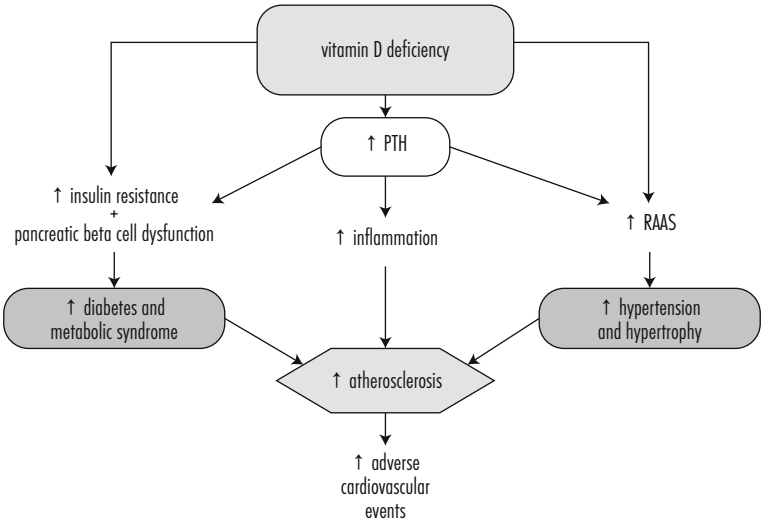

Figure 28.1. Proposed mechanisms of vitamin D deficiency-related cardiovascular risk (Lee *et al.*, 2008; reprinted with permission).

correlated only weakly (r=-0.15) with 25(OH)D, with both PTH and 25(OH)D contributing independently to CV risk and disease prediction (Anderson *et al.*, 2011). Similarly, Kestenbaum *et al.* (2011) found vitamin D and PTH to contribute independently to CV risk.

One proposed non-PTH related CVD mechanism is an association with large high-density lipoprotein particles (Kazlauskaite *et al.*, 2010). Further, $1,25(OH)_2D$ has been reported to inhibit foam cell formation and suppress macrophage cholesterol uptake in patients with type 2 diabetes (Oh *et al.*, 2009). Additional recent evidence suggests that vitamin D deficiency may lead to endothelial dysfunction and arterial stiffness and that supplementation may improve vascular function (McGreevy and Williams, 2011). Other molecular mechanisms of CVD likely await discovery.

To determine whether the vitamin D deficiency-related increase in coronary risk is associated with progression of atherosclerosis or with plaque instability, we measured 25(OH)D levels in 3,413 consecutive patients participating in the Intermountain Heart Collaborative Study Registry who were undergoing coronary angiography (May *et al.*, 2010). No association was found between the severity of angiographic CAD and vitamin D status, whereas in the same population, vitamin D deficiency was associated with an increase in incident CV events, including MI. These findings support the hypothesis that vitamin D plays a role in plaque stability but not in plaque growth.

28.8 Reported associations between vitamin D deficiency and cardiovascular risk

Primarily within the past 5-8 years, important CV risk associations with vitamin D deficiency have been forthcoming. The first important CVD associations emerged from observations in patients with end-stage chronic kidney disease on dialysis. Two-fold increases in CV mortality and total mortality were noted in those with 25(OH)D levels <10 ng/ml, whereas active vitamin D therapy was associated with a substantially reduced mortality risk (Wolf *et al.*, 2007). A pair of studies in the Archives of Internal Medicine in 2008 associated vitamin D deficiency with CV risk: Giavanucci *et al.* (2008) found an association of 25(OH)D deficiency and risk of MI in men. Dobnig *et al.* (2008) reported that low 25(OH)D and $1,25(OH)_2D$ levels independently were associated with all-cause and CV mortality in a cohort study of 3,258 men and women scheduled for coronary angiography. Hazard ratios for quartiles 1 (median 25(OH)D 7.6 ng/ml) and 2 (median 25(OH)D 13.3 ng/ml) for CV death were 2.2 and 1.8, respectively (both $P<0.01$), compared to quartile 4 (median 25(OH)D 28.4 ng/ml). A cross-sectional study examined the burden of CVD associated with hypovitaminosis D using data from the 2001-2004 NHANES. The prevalence of 25(OH)D <30 ng/ml in 8,351 adults with measured levels was 74% (Kim *et al.*, 2008). The burden of all CVD, and coronary heart disease, heart failure, stroke, and peripheral vascular disease, increased (by 1.5 to 2 fold) with decreasing 25(OH)D categories (≥30, 20-29, <20 ng/ml). In a Framingham offspring study, vitamin D deficiency (≤15 ng/ml) was found to increase the risk of incident CV events during 7 years of follow-up by 2-2.5 fold, with an especially large absolute increment in risk in hypertensive subjects (Wang *et al.*, 2008).

Stimulated by these initial reports, we undertook a prespecified analysis of data within Intermountain Healthcare's eMR database (Anderson *et al.*, 2010). We identified 41,497 patients in whom at least one serum 25(OH)D level was measured (levels were drawn at healthcare providers' discretion for the usual indications). The distribution of 25(OH)D levels was found to be >30 ng/ml in 36%, 16-30 ng/ml in 47%, and ≤15 ng/ml in 17%. The baseline prevalence of hyperlipidemia and especially hypertension (39.8% to 43.8% to 51.9%) and diabetes (15.4% to 20.0% to 29.3%) increased in stepwise fashion with decreasing serum 25(OH)D level category in the cohort of subjects 50 years of age or older (n=27,686) evaluated for CV conditions (all $P<0.001$). During subsequent follow-up (average 1.3, maximum 9.3 years), the risk of new onset (incident) hyperlipidemia (HR 1.27, $P=0.003$) and especially hypertension (HR 1.62, $P<0.001$) and diabetes (HR 1.89, $P<0.001$) was increased in those with very low (≤15 ng/ml) compared to those with normal levels (Table 28.2). Similarly, a low level of 25(OH)D was associated with CVD outcomes, including incident CAD/MI and especially heart failure, stroke, and death (Table 28.3); survival curves by 25(OH)D category are shown in Figure 28.2.

Table 28.2. Incidence of cardiovascular risk factors by baseline 25(OH)D levels in those without prior condition (reprinted from Anderson *et al.*, 2010).

Risk factor (overall incidence)	Very low vs. normal (≤15 vs. >30 ng/ml)		Low vs. normal (16-30 vs. >30 ng/ml)	
	Unadjusted	Adjusted	Unadjusted	Adjusted
Hypertension (6.0%)				
Hazard ratio	1.73	1.62	1.26	1.18
95% CI	1.48-2.02	1.38-1.89	1.12-1.42	1.05-1.33
P-value	<0.0001[a]	<0.0001[a]	<0.0001[a]	0.005[a]
Hyperlipidemia (5.8%)				
Hazard ratio	1.47	1.27	1.19	1.10
95% CI	1.25-1.72	1.09-1.50	1.06-1.34	0.98-1.24
P-value	<0.0001[a]	0.003[a]	0.005[a]	0.12
Diabetes mellitus (2.2%)				
Hazard ratio	2.13	1.89	1.39	1.32
95% CI	1.73-2.62	1.54-2.33	1.17-1.64	1.12-1.56
P-value	<0.0001[a]	<0.0001[a]	<0.0001[a]	0.001[a]
Peripheral vascular disease (0.8%)				
Hazard ratio	1.80	1.42	1.10	1.01
95% CI	1.32-2.46	1.04-1.94	0.85-1.43	0.78-1.31
P-value	<0.0001[a]	0.03	0.48	0.93

Follow-up averaged 1.3 years (maximum, 9.3).

[a] Comparisons also significant after Bonferroni correction (i.e. adjusted for 8 risk factor comparisons).

Table 28.3. 25(OH)D levels and incident cardiovascular diagnoses and mortality. Outcomes are adjusted for multiple baseline characteristics (Anderson *et al.*, 2010).

Outcomes (patients ≥50 years old)	≤15 vs. >30 ng/ml	16-30 vs. >30 ng/ml
Death (n=27,686)	HR=1.77, *P*<0.0001	HR=1.20, *P*=0.009
CAD/MI (n=21,853)[1]	HR=1.45, *P*<0.0001	HR=1.15, *P*=0.09
Heart failure (n=23,793)	HR=2.01, *P*<0.0001	HR=1.31, *P*=0.005
Stroke (n=26,025)	HR=1.78, *P*=0.004	HR=1.31, *P*=0.11
Atrial fibrillation (n=24,565)	HR=1.02, *P*=0.87	HR=0.95, *P*=0.61

[1] CAD = coronary artery disease; MI = myocardial infarction. Numbers (n) represent population database with information available for 25(OH)D levels and each diagnosis of interest.

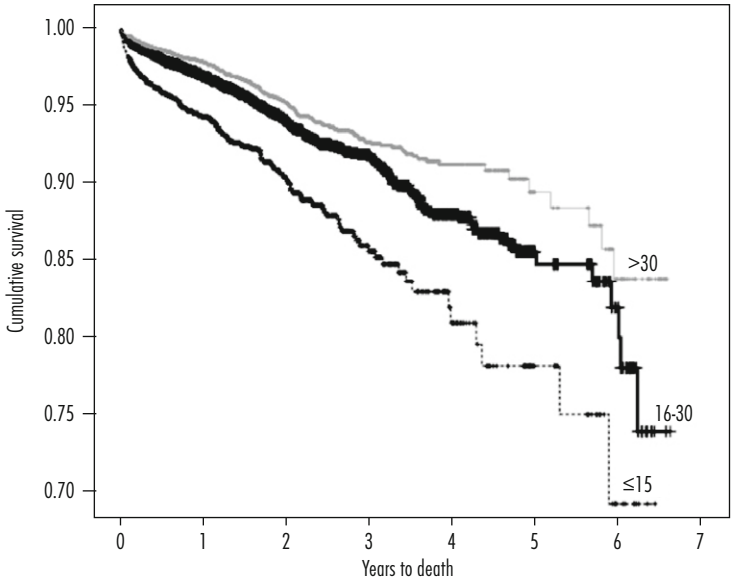

Figure 28.2. Survival by initial 25(OH)D levels (Anderson *et al.*, 2010; reprinted with permission).

In support, a review of the literature relating to vitamin D and CV risk through February 2012 by Wang *et al.* (2012) identified 19 studies involving 6,123 CV disease cases in 65,994 subjects. Comparing the highest with lowest vitamin D quantiles, vitamin D deficiency was associated with a RR of total CVD of 1.53, CV mortality RR=1.44, coronary heart disease RR=1.38, and stroke RR=1.64. CVD risk increased monotonically with decreasing 25(OH)D levels <75 nmol/l (30 ng/ml) by RR=1.03-1.07 for each 25 nmol/l (10 ng/ml) decrement in 25(OH)D.

28.9 Can vitamin D supplementation reduce cardiovascular risk?

Trials of dietary supplementation with several other vitamins and nutrients to improve general and CV health generally have a dismal record, with multiple failures. As questioned by one editorialist: 'Why vitamin D if not vitamin A, B, C, or E?' (Byers, 2010) One might add folic acid and, given vitamin D's actions as a hormone, hormone (estrogen/progestin) replacement therapy. An axiom of essential nutrients has been used as an argument in favor of vitamin D as an exception to this otherwise generally negative experience: supplementing a normal nutrient level to a supra-physiologic range may result in neutral to harmful effects, whereas repleting a deficiency may confer benefit. In affluent Western societies, true deficiencies of vitamins A, B, C, and E, and folic acid (given current food supplementation), are rare. In contrast, the prevalence of low to very low levels of vitamin D is common and increasing. Nevertheless, given this generally negative experience with intervention trials with other vitamins, a healthy skepticism is appropriate in considering the potential of vitamin D supplementation to reduce CV risk. Observational data and controlled trials results for vitamin D supplementation currently are quite limited and do not provide a definitive answer for CV prevention.

In a historical cohort study in a large dialysis database, Teng *et al.* (2005) observed that IV vitamin D supplementation (n=37,173), compared to no supplementation (n=13,864), was associated with half the rate of overall mortality (13.8% vs. 26.8%) and CV mortality (7.6% vs. 14.6%). Similarly, Wolf *et al.* (2007) observed low all-cause and CV mortality in a dialysis patient cohort that was treated with active vitamin D therapy. In a 2007 report, Autier and Gandini (2007) summarized results of 9 randomized studies of differing size, design, study populations, and with generally limited numbers of events, in which patients received vitamin D supplements or control therapy. A modest survival benefit (relative risk 0.92) was suggested. In a more recent meta-analysis, which included 17 prospective cohort and randomized studies, Wang *et al.* (2012) found that moderate to high supplemental doses of vitamin D may reduce the risk of CV disease, with benefit primarily seen in dialysis patients.

While awaiting prospective randomized trials, we undertook a quasi-intervention trial based on Intermountain healthcare eMR records data for 7,515 adults with an initial and at least one follow-up 25(OH)D level at least one year apart (Bair *et al.*, 2010). The last follow-up level used was either the first level when 25(OH)D was normalized (≥30 ng/ml) or the last level obtained. Subsequent patient follow-up averaged 2.5 years (maximum, 5.5 y). Cox regression was used to compare survival in those with normalized (presumably by supplementation) or not, adjusted for outcomes of death, incident myocardial infarction or a coronary artery disease diagnosis, heart failure, stroke, or clinical renal failure. Normalization of vitamin D was associated with reduced hazards of composite CV events (HR=0.80, $P<0.05$), death (HR=0.77, $P<0.05$), CAD (HR=0.85, $P<0.05$), MI (HR=0.75), heart failure (HR=0.83, $P<0.05$), and renal failure (HR=0.76, $P<0.05$), with no effect on stroke (HR=0.97). The survival curve for the composite outcome is shown in Figure 28.3.

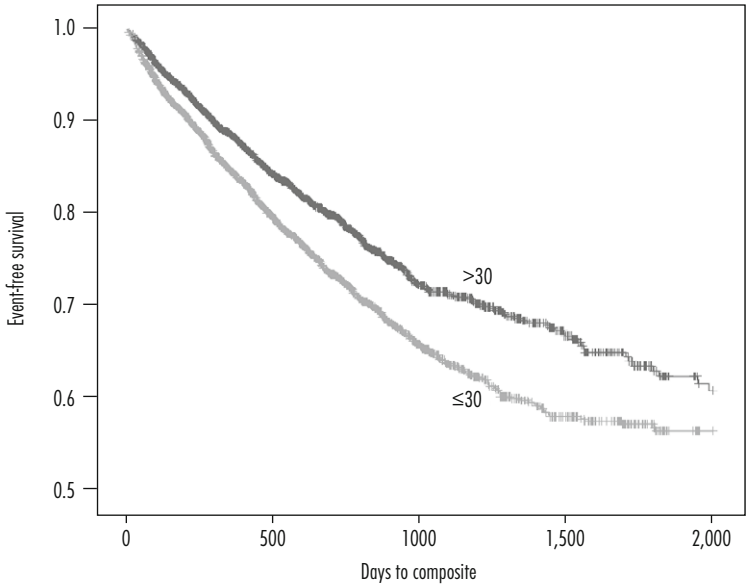

Figure 28.3. Survival curves for composite cardiovascular outcomes in those with and without normalization of 25(OH)D (ng/ml) during follow-ups (Bair *et al.*, 2010).

VITAL (*VIT*amin D and Omeg*A*-3 Tria*L)* represents a major intervention trial of vitamin D and omega-3 fatty acid supplementation. Funded by the National Institutes of Health and coordinated by Harvard Medical School/Brigham and Women's Hospital, VITAL was launched in January 2010 with a target enrollment of 20,000. Entry requirements are ages ≥60 in men, ≥65 in women, no history of cardiac disease or cancer, and not taking major supplemental doses of vitamin D (>800 U/day) or calcium (>1,200 mg/d). Interventions include vitamin D3 2,000 U daily (or placebo) and omega-3 fatty acids (Omacor® fish oil) 1000 mg daily (or placebo). The primary objectives of VITAL are to determine whether vitamin D or omega-3 fatty acids can prevent cancer, heart disease, and stroke. However, design issues in VITAL (e.g. inclusion of a general population rather than those selected for true deficiency, and insufficient supplementation) may leave the question of whom/how to treat unanswered, and other trials may be required, and other intervention trials of vitamin D supplementation are in planning or enrollment stages.

28.10 Not all the evidence is positive

Not all recent evidence has been supportive of either CV disease associations or the benefits of vitamin D supplementation. A systematic review of 13 observational studies reported that the associations between vitamin D status and cardiometabolic outcomes (i.e. hypertension, diabetes, CV disease) were heterogeneous and the implications uncertain (Pittas *et al.*, 2010). Another recent overview included 51 trials and reported that the evidence that vitamin D status and vitamin D supplementation could be used to reduce CV risk and mortality was inconclusive (Elamin *et*

al., 2011). Low dose vitamin D plus calcium supplements did not improve CV outcomes in post-menopausal women in the Women's Health Initiative (Hsia *et al.*, 2007). Among patients with chronic kidney disease, 48 weeks of supplementation with paricalcitrol did not improve measures of LV mass or diastolic function (Thadani *et al.*, 2012).

28.11 Pending prospective trials, empiric therapy or treat to target?

The recent IOM report for population-based vitamin D supplementation to achieve adequate 25(OH)D levels (defined as >20 ng/ml) recommended 600 IU daily for ages ≤70 and 800 IU daily for ages >70 (Ross *et al.*, 2011). These doses were projected to meet minimal requirements for at least 97.5% of the population. In support, a placebo-controlled randomized trial in 163 post-menopausal women found that supplementation with 800 IU/d of vitamin D3 achieved 25(OH) D levels of ≥50 nmol/l (20 ng/ml) in 97.5% of women (Gallagher *et al.*, 2012). Pending additional clinical trials data, this general empiric approach to overall health maintenance (including CV health) is reasonable.

An alternative interim approach is to measure baseline 25(OH)D levels in subjects at risk of vitamin D deficiency and its associated morbidities and supplement to greater than a minimum target serum 25(OH)D level, (i.e. >20-30 ng/ml, and <50-70 ng/ml). Some of the conditions predisposing to vitamin D deficiency include: darker skin; indoor lifestyle, sun avoidance; high latitudes; smoking, air pollution; advanced age, especially with other risk factors or conditions; bone/muscle/joint complaints (myalgias, arthralgias, osteopenia, osteoporosis); obesity, diabetes; hypertension, coronary disease, heart failure; hospitalized, institutionalized, and home-bound persons, nursing home residents; infectious diseases, immune deficiencies; chronic kidney or liver disease (McGreevy and Williams, 2011).

28.12 Testing for vitamin D, costs and coverage

Vitamin D currently is one of the most commonly ordered 'esoteric tests'. A typical outpatient laboratory charge for serum 25(OH)D ranges widely from $50-$300. On-line or wholesale prices may be as low as $22-$35. Medicare reimbursement at the time of this writing is $43, but reimbursement is limited to specific traditional indications, including myalgias, disorders of bone and cartilage, osteoporosis, renal insufficiency, and vitamin D deficiency. CV risk assessment and CV disorders/diseases are not covered. Coverage by individual private insurance companies varies, but many follow Medicare guidelines. These cost and reimbursement considerations should be kept in mind when considering 25(OH)D measurements for the purpose of CV risk assessment.

28.13 Supplementing vitamin D

With a goal of supplementing deficient 25(OH)D levels to >20-30 ng/ml (but to <50 ng/ml? (Ross *et al.*, 2011)), vitamin D supplements are available as vitamin D2 (ergocalciferol), a plant-based form, and vitamin D3 (cholecalciferol), an animal-based and generally preferred form. If following general IOM recommendations, daily doses are 600 IU for ages ≤70 and 800 IU for ages >70. For treatment of identified vitamin D deficiency (i.e. <10-20 ng/ml), initial 'loading' may be given as 5,000 IU/d for 2-4 months, followed by an initial maintenance dose of 1000-2,000 IU daily with retesting to establish achievement of target levels (Holick, 2007; Kennel *et al.*, 2010). The IOM report gives 4,000 IU/d in adults as the upper dosing limit to avoid potential adverse effects (Ross *et al.*, 2011).

28.14 Can too much vitamin D be harmful?

The IOM report concluded that available scientific evidence supports a key role of calcium and vitamin D in skeletal health, consistent with a cause-and-effect relationship, but found that for extraskeletal conditions, including CV diseases, diabetes, and cancer, the evidence was insufficient and inconclusive enough to inform specific vitamin D nutritional requirements (Ross *et al.*, 2011). Further, their review suggested that higher values were not consistently associated with greater benefit and that for some outcomes a U-shaped association appeared to exist (including CV disease, vascular calcifications, frailty/falls, pancreatic cancer, and all-cause mortality) such that increased risk was observed at both high and low levels, with levels >50 ng/ml raising concerns (Melamed *et al.*, 2008; Ross *et al.*, 2011; Wang *et al.*, 2008).

Evidence for a vascular pro-inflammatory response, as determined by high-sensitivity C-reactive protein levels in an NHANES population, was recently reported at both low (<20 ng/ml) and also high (>50 ng/ml) 25(OH)D levels (Amer and Qayyum, 2012). In our Intermountain Healthcare database, an increased risk of atrial fibrillation was suggested at high (>100 ng/ml) 25(OH)D levels (Smith *et al.*, 2011).

In contrast, frank vitamin D toxicity is rare and is typically manifested by symptoms of hypercalcemia. Most reports of vitamin D toxicity have been associated with 25(OH)D levels of 150 ng/ml or greater, with 80 ng/ml being the lowest reported level associated with toxicity (Jones, 2008; Kennel *et al.*, 2010).

28.15 Vitamin D and cardiovascular risk: conclusions

Beyond bone health, the role of vitamin D in general health and a broad spectrum of common disorders has been the focus of great interest in the past few years, and a rapid growth in our knowledge base about vitamin D has occurred, but major gaps remain. Among new areas of interest is the role of vitamin D in CV health and disease. Emerging science indicates that vitamin

D is a hormone that acts on receptors in multiple organs and tissues. Vitamin D originates primarily by synthesis in the skin in response to sun exposure, and deficiency is a common consequence of our modern lifestyle in the general population and in CV patients. Growing evidence associates vitamin D deficiency with CV risk factors and incident CVD. Observational evidence generally supports the use of vitamin D supplementation in truly deficient patients to reduce CV risk, but the evidence is inconclusive, and randomized trials are needed and are underway. However, a 'U-shaped' benefit-risk association appears to exist for vitamin D, as for other vitamins/supplements, so that repletion of deficiency, but not supplementation to supra-normal levels, should be targeted. Currently, either an empiric approach to lifestyle and supplementation or selected screening and treatment to target may be considered pending more definitive clinical trials evidence. In summary, more robust evidence is needed to conclusively demonstrate that vitamin D supplementation can play a major role in reducing CV risk factors and in preventing or treating CVD. So, is vitamin D mostly hype or a new hope for CV prevention (Lavie *et al.*, 2011)? It probably is some of each, which ongoing and future research must clarify (Lavie *et al.*, 2011).

References

Amer, M. and Qayyum, R., 2012. Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults American Journal of Cardiology 109, 226-230.

Anderson, J.L., May, H.T., Horne, B.D., Bair, T.L., Hall, N.L., Carlquist, J.L., Lappe, D.L. and Muhlestein, J.B., 2010. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. American Journal of Cardiology 106, 963-968.

Anderson, J.L., Vanwoerkom, R.C., Horne, B.D., Bair, T.L., May, H.T., Lappé, D.L. and Muhlestein, J.B., 2011. Parathyroid hormone, vitamin D, renal dysfunction, and cardiovascular disease: dependent or independent risk factors. American Heart Journal 162, 331-339.

Autier, P. and Gandini, S., 2007. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Archives of Internal Medicine 167, 1730-1737.

Bair, T., Muhlestein, J.B., May, H.T., Horne, B.D., Carlquist, J.F., Lappe, D.L. and Anderson, J.L., 2010. Supplementing deficient vitamin D levels is associated with reduced cardiovascular risk. Journal of the American College of Cardiology 55, A59 (1186-1111).

Byers, T., Anticancer vitamins du jour – The ABCED's so far. American Journal of Epidemiology 172, 1-3.

Dawson-Hughes, B., Mithal, A., Bonjour, J.P., Boonen, S., Burckhardt, P., Fuleihan, G.E., Josse, R.G., Lips, P., Morales-Torres, J. and Yoshimura, N., 2010. IOF position statement: vitamin D recommendations for older adults. Osteoporosis International 21, 1151-1154.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., Kinkeldei, J., Boehm, B.O., Weihrauch, G. and Maerz, W., 2008. Independent association of low serum 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Archives of Internal Medicine 168, 1340-1349.

Elamin, M.G., Abu Elnour, N.O., Elamin, K.B., Fatourechi, M.M., Alkatib, A.A., Almandoz, J.P., Liu, H., Lane, M.A., Mullan, R.J., Hazem, A., Erwin, P.J., Hensrud, D.D., Murad, M.H. and Montori, V.M., 2011. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. Journal of Clinical Endocrinology and Metabolism 96, 1931-1942.

Gallagher, J.C., Sal, A., Templin, T. and Smith, L., 2012. Dose response to vitamin D supplementation in post menopausal women. Annals of Internal Medicine 126, 425-437.

Giovannucci, E., Liu, Y., Hollis, B.W. and Rimm, E.B., 2008. 25-Hydroxyvitamin D and risk of myocardial infarction in men. Archives of Internal Medicine 168, 1174-1180.

Holick, M.F., 2007. Vitamin D deficiency. New England Journal of Medicine 357, 266-281.

Hsia, J., Heiss, G., Ren, H., Allison, M., Dolan, N.C., Greenland, P., Heckbert, S.R., Johnson, K.C., Manson, J.E., Sidney, S., Trevisan, M. and the women's health initiative investigators, 2007. Calcium/vitamin D supplementation and cardiovascular events. Circulation 115, 846-854.

Jones, G., 2008. Pharmacokinetics of vitamin D toxicity. American Journal of Clinical Nutrition 88, 582S-586S.

Kazlauskaite, R., Powell, L.H., Mandapakala, C., Cursio, J.F., Avery, E.F. and Calvin, J., 2010. Vitamin D is associated with atheroprotective high-density lipoprotein profile in postmenopausal women. Journal of Clinical Lipidology 4, 113-119.

Kennel, K.A., Drake, M.T. and Hurley, D.L., 2010. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clinic Proceedings 85, 752-758.

Kestenbaum, B., Katz, R., de Boer, I., Hoofnagle, A., Sarnak, M.J., Shlipak, M.G., Jenny, N.S. and Siscovick, D.S.,, 2011. Vitamin D, parathyroid hormone, and cardiovascular events among older adults. Journal of the American College of Cardiology 58, 1433-1441.

Kim, D.H., Sabour, S., Sagar, U.N., Adams, S. and Whelan, D.J., 2008. Prevalence of hypovitaminosis D in cardiovascular diseases. American Journal of Cardiology 102, 1540-1544.

Lavie, C.J., Lee, J.H. and Milani, R.V., 2011. Vitamin D and cardiovascular disease: will it live up to its hype? Journal of the American College of Cardiology 58, 1447-1456.

Lee, J.H., O'Keefe, J.H., Bell, D., Hensrud, D.D. and Holick, M.F., 2008. Vitamin D deficiency: an important, common, and easily treatable cardiovascular risk factor? Journal of the American College of Cardiology 52, 1949-1956.

Lips, P., 2001. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consdequences for bone loss and fractures and therapeutic implications. Endocrine Reviews 22, 477-501.

May, H.T., Bair, T.L., Galenko, O., Horne, B.D., Lappe, D.L., Carlquist, J.L., Ronnow, B.S., Gunter, S., Anderson, J.L. and Muhlestein, J.B., 2010. No association between baseline vitamin D levels and angiographic severity of coronary artery disease. Circulation 122, A16033.

McGreevy, C. and Williams, D., 2011. New insights about vitamin D and cardiovacular disease. Annals of Internal Medicine 155, 820-826.

Melamed, M.L., Michos, E.D., Post, W. and Astor, B.,2008. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Archives of Internal Medicine 168, 1629-1637.

Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhardt, P., Dawson-Hughes, B., Eisman, J.A., El-Hajj Fuleihan, G., Josse, R.G., Lips, P., Morales-Torres, J. and the IOF Committee of Scientific Advisors (CSA) Nutrition Working Group, 2009. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International 20, 1807-1820.

Oh, J., Weng, S., Felton, S.K., Bhandare, S., Riek, A., Butler, B., Proctor, B.M., Petty, M., Chen, Z., Schechtman, K.B., Bernal-Mizrachi, L. and Bernal-Mizrachi, C., 2009. 1,25(OH)2 Vitamin D inhibits foam cell formation and suppresses macrophage cholelsterol uptake in patients with type 2 diabetes mellitus. Circulation 120, 687-698.

Pittas, A.G., Chung, M., Trikalinos, T., Mitri, J., Brendel, M., Patel, K., Lichtenstein, A.H., Lau, J. and Balk, E.M., 2010. Systematic review: vitamin D and cardiometabolic outcomes. Annals of Internal Medicine 152, 307-314.

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Benjamin, E.J., Berry, J.D., Borden, W.B., Bravata, D.M., Dai, S., Ford, E.S., Fox, C.S., Fullerton, H.J., Gillespie, C., Hailpern, S.M., Heit, J.A., Howard, V.J., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Makuc, D.M., Marcus, G.M., Marelli, A., Matchar, D.B., Moy, C.S., Mozaffarian, D., Mussolino, M.E., Nichol, G., Paynter, N.P., Soliman, E.Z., Sorlie, P.D., Sotoodehnia, N., Turan, T.N., Virani, S.S., Wong, N.D., Woo, D., Turner, M.B. and the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, 2012. Executive summary: heart disease and stroke statistics – 2012 update. Circulation 125, 188-197.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 report on dietary reference intakes for calciuym and vitamin D from the Institute of Medicine: what clinicians need to know. Journal of Clinical Endocrinology and Metabolism 96, 53-58.

Shirts, B.H., Howard, M.T., Hasstedt, S.J., Nanjee, M.N., Knight, S., Carlquist, J.L., Anderson, J.L., Hopkins, P.N. and Hunt, S.C., 2012. Vitamin D dependent effects of ApoA5 polymorphisms on HDL cholesterol. Atherosclerosis 222, 167-174.

Smith, M.B., May, H.T., Bair, T.L., Anderson, J.L., Anderson, J.L., Muhlestein, J.B., Horne, B.D., Lappe, D.L., Day, J.D., Crandall, B.G., Weiss, P., Osborn, J.S. and Bunch, T.J., 2011. Vitamin D excess is significantly associated with risk of atrial fibrillation. Circulation 122, (suppl): A14699.

Tangpricha, V., Pearce, E.N., Chen, T.C. and Holick, M.I., 2002. Vitamin D insufficiency among free-living healthy young adults. American Journal of Medicine 112, 659-662.

Teng, M., Wolf, M., Ofsthun, M.N., Lazarus, J.M., Hernán, M.A., Camargo Jr., C.A. and Thadhani, R., 2005. Activated injectable vitamin D and hemodialysis: a historical cohort study. Journal of the American Society of Nephrology 16, 1115-1125.

Thadani, R., Appelbaum, E., Pritchett, Y., Chang, Y., Wenger, J., Tamez, H., Bhan, I., Agarwal, R., Zoccali, C., Wanner, C., Lloyd-Jones, D., Cannata, J., Thompson, B.T., Andress, D., Zhang, W., Packham, D., Singh, B., Zehnder, D., Shah, A., Pachika, A., Manning, W.J. and Solomon, S.D., 2012. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease. JAMA 307, 674-684.

Wallis, D.E., Penckofer, S. and Sizemore, G.W., 2008. The "sunshine deficit" and cardiovascular disease. Circulation 118, 1476-1485.

Wang, L., Song, Y., Manson, J.E., Pilz, S., Marz, W., Michaelsson, K., Lundqvist, A., Jassal, S.K., Barrett-Connor, E., Zhang, C., Eaton, C.B., May, H.T., Anderson, J.L. and Sesso, H.D., 2012. Circulating 25-hydroxy-vitamin d and risk of cardiovascular disease: a meta-analysis of prospective studies. Circulation: Cardiovascular Quality and Outcomes 5, 819-829.

Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M. and Vasan, R.S.,2008. Vitamin D deficiency and risk of cardiovascular disease. Circulation 117, 503-511.

Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., van Meurs, J.B., Berry, D., Kiel, D.P., Streeten, E.A., Ohlsson, C., Koller, D.L., Peltonen, L., Cooper, J.D., O'Reilly, P.F., Houston, D.K., Glazer, N.L., Vandenput, L., Peacock, M., Shi, J., Rivadeneira, F., McCarthy, M.I., Anneli, P., de Boer, I.H., Mangino, M., Kato, B., Smyth, D.J., Booth, S.L., Jacques, P.F., Burke, G.L., Goodarzi, M., Cheung, C.L., Wolf, M., Rice, K., Goltzman, D., Hidiroglou, N., Ladouceur, M., Wareham, N.J., Hocking, L.J., Hart, D., Arden, N.K., Cooper, C., Malik, S., Fraser, W.D., Hartikainen, A.L., Zhai, G., Macdonald, H.M., Forouhi, N.G., Loos, R.J., Reid, D.M., Hakim, A., Dennison, E., Liu, Y., Power, C., Stevens, H.E., Jaana, L., Vasan, R.S., Soranzo, N., Bojunga, J., Psaty, B.M., Lorentzon, M.,

Foroud, T., Harris, T.B., Hofman, A., Jansson, J.O., Cauley, J.A., Uitterlinden, A.G., Gibson, Q., Järvelin, M.R., Karasik, D., Siscovick, D.S., Econs, M.J., Kritchevsky, S.B., Florez, J.C., Todd, J.A., Dupuis, J., Hyppönen, E. and Spector, T.D., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180-188.

Wolf, M., Shah, A., Gutierrez, O., Ankers, E., Monroy, M., Tamez, H., Steele, D., Chang, Y., Camargo Jr, C.A., Tonelli, M. and Thadhani, R., 2007. Vitamin D levels and early mortality among incident hemodialysis patients. Kidney International 72, 1004-1013.

Yusuf, S., Reddy, S., Ounpuu, S. and Anand, S., 2001. Global burden of cardiovascular disease, part II. Circulation 104, 2855-2864.

Zitterman, A., 2006. Vitamin D and disease prevention with special reference to cardiovascular disease. Progress in Biophysics and Molecular Biology 92, 39-48.

Key facts

- It is important to correct 25(OH)-vitamin D (25(OH)D) insufficiency after transplantation since 25(OH)D does not only serve as a substrate for 1α-hydroxylase in the kidney but also in several extra-renal tissues.
- Vitamin D insufficiency is frequent in transplant recipients (TR) and concerns currently more than 85% of adult renal TR.
- Increased 25(OH)D catabolism induced by immunosuppressive drugs and sun exposure avoidance recommended to TR may explain the frequency of vitamin D insufficiency after transplantation and the need for high dosage of native vitamin D in this population.
- In the field of transplantation, experimental studies focused on the potential protective role for vitamin D against acute rejection and clinical studies are mainly observational, except for those concerning prevention of bone loss and two small studies concerning immunomodulatory effects of calcitriol after renal transplantation.
- Two randomized control studies are actually undergoing to test the effect of cholecalciferol after renal transplantation on patients and renal grafts' outcomes.

Summary points

- Vitamin D has been shown to play an important role in reducing risk of many chronic diseases including osteoporosis, type 2 diabetes mellitus (DM), cardiovascular diseases, cancers and to have immunomodulatory effects.
- Due to specific and non-specific risk factors, TR are at high risk of many diseases such as osteoporosis, type 2 DM, cardiovascular diseases, cancers, and acute graft rejection.
- Although vitamin D supplementation has been studied after renal transplantation, the optimal scheme for vitamin D supplementation remains to be determined after lung, liver and cardiac transplantation.
- Immediately after transplantation, randomized trials showed that active form of vitamin D could prevent or attenuate lumbar spine and femoral neck bone loss.
- After renal transplantation, low serum 25(OH)D correlates with hyperparathyroidism and efficient treatment of vitamin D insufficiency with cholecalciferol was shown to reduce serum parathormone concentration in renal TR.
- No study reported the potential effect of activated or native vitamin D on *de novo* type 2 DM or on cardiovascular risk after transplantation.
- A higher incidence of post-transplant cancers was observed in patients with pre-transplant 25(OH)D concentrations less than 10 ng/ml but this result remains to be confirmed in an interventional randomized trial.
- Many experimental data showed that vitamin D could prolong graft survival or prevent acute rejection in animal models without increasing the risk of fungal or viral infection and two small interventional studies in TR confirmed the immunomodulatory effect of active vitamin D after transplantation.
- A number of clinical studies have confirmed the potent renoprotective and antiproteinuric effect of vitamin D but this point remains to be confirmed in TR.

29. Vitamin D and transplantation

M. Courbebaisse[1], J.C. Souberbielle[2] and E. Thervet[3]
[1]Service de Physiologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France; [2]Service de Physiologie, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75015 Paris, France; [3]Service de Néphrologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France; marie.courbebaisse@egp.aphp.fr

Abstract

Mainly due to immunosuppressive treatments and sun exposure avoidance, transplant recipients are at high risk of vitamin D insufficiency (25(OH)-vitamin D<30 ng/ml). Besides its major role for bone health, vitamin D has been shown to play an important role in reducing risk of many chronic diseases including cancers, type 2 diabetes mellitus, chronic kidney disease, cardiovascular diseases and infectious diseases. All these diseases are more likely to occur in transplant recipients, who have induced immunodeficiency, than in the general population. It is therefore of great importance to correct vitamin D insufficiency after transplantation as 25(OH)-vitamin D does not only serve as a substrate for 1α-hydroxylase in the kidney but also in several extra-renal tissues. The pleiotropic effects of vitamin D are mostly documented by observational and experimental studies or by small interventional trials that most often evaluated intermediate parameters. In the field of transplantation, experimental studies focused on the potential protective role for vitamin D against acute graft rejection and clinical studies are mainly observational, except for those concerning prevention of bone loss. Indeed, immediately after transplantation, randomized trials showed that active form of vitamin D could prevent or attenuate lumbar spine and femoral neck bone loss. However, randomized interventional trials are still needed before one may consider that non classical effects of vitamin D that, for most, are especially relevant for the care of transplant patients, really apply to transplant recipients. Actually, two randomized interventional trials are undergoing to test the effects of high doses of cholecalciferol after renal transplantation on: (1) graft function, incidence of acute rejection episodes and post-transplant infections within the first year after transplantation for VITA-D study; (2) a composite endpoint consisting in de novo diabetes mellitus, cardiovascular diseases, de novo cancer and patient death after the first year post-transplantation for VITALE study.

Keywords: graft, acute rejection, immunosuppressive treatment

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_29, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
AR	Acute rejection
CYP27B1	Gene encoding for 1α-hydroxylase
DCs	Dendritic cells
DM	Diabetes mellitus
EMT	Epithelial-to-mesenchymal transition
IL	Interleukin
KO	Knockout
MMP	Matrix metalloproteinase
MT	*Mycobacterium tuberculosis*
NFκB	Nuclear factor-kappa B
PTH	Parathyroid hormone
RAS	Renin-angiotensin system
TGF-β	Transforming growth factor-β
TR	Transplant recipients
UVB	Ultraviolet B
VDR	Vitamin D receptor
VDRE	Vitamin D response element

29.1 Introduction

Traditionally, vitamin D has been associated with bone health: vitamin D deficiency leads to rickets in children and osteomalacia in adults and increases the risk of osteoporosis. More recently, vitamin D has been shown to play an important role in reducing risk of many chronic diseases including type 2 DM, cardiovascular diseases, cancers and to have immunomodulatory effects. All these diseases are more likely to occur in TR than in the general population. Moreover, vitamin D insufficiency is a frequent finding in TR, due to multiple causes, including immunosuppressive treatments and sun exposure avoidance. Although the data presented here were generally obtained in non-transplant patients, this chapter will put into perspective the potential actions of vitamin D in TR. However, we must acknowledge that intervention trials are needed before one may consider that these effects really apply to TR.

29.2 Vitamin D insufficiency after transplantation

Especially after kidney transplantation, vitamin D in its active form is currently used for the prevention of post-transplant bone loss (Palmer *et al.*, 2007) and in the treatment of normocalcemic persistent secondary hyperparathyroidism (De Sevaux *et al.*, 2002; El-Agroudy *et al.*, 2003; Lobo *et al.*, 1995; Torres *et al.*, 2004). However, one has to keep in mind, as suggested in a recent review (Thiem and Borchhardt, 2011), that treatment with active vitamin D and its

analogues will not compensate for inadequate 25(OH)D status. It is thus still important to correct 25(OH)D insufficiency since 25(OH)D does not only serve as a substrate for 1α-hydroxylase in the kidney but also in several extra-renal tissues. Hence, these extra-renal tissues are dependent on adequate 25(OH)D substrate for adequate local calcitriol production. Although there is no current consensus, vitamin D insufficiency is defined as 25(OH)D levels lower than 30 ng/ml (or 75 nmol/l) (Dawson-Hughes *et al.*, 2005) because this limit is associated with a decrease in active calcium intestinal absorption (Heaney, 2003) and an increase in PTH secretion aiming at maintaining normal serum calcium level (Dawson-Hughes *et al.*, 2005). Furthermore, in interventional studies showing positive effects of vitamin D supplementation, the 25(OH)D levels reached in the treated groups were generally higher than 30 ng/ml (Bischoff-Ferrari *et al.*, 2006). Regarding the upper limit, vitamin D intoxication does not occur if 25(OH)D concentration remains less than 150 ng/ml (Vieth *et al.*, 2001; Zittermann, 2003).

Vitamin D insufficiency is frequent in TR and concerns currently more than 85% of adult renal TR (Querings *et al.*, 2006; Sadlier and Magee, 2007; Stavroulopoulos *et al.*, 2007). Vitamin D insufficiency is also frequent in paediatric renal TR (Shroff *et al.*, 2011; Sgambat *et al.*, 2011). Moreover, vitamin D insufficiency is also frequently found after heart, liver or lung transplantation (Stein *et al.*, 2009; Segal *et al.*, 2001; Lowery *et al.*, 2012). Various causes may be implicated: (1) insufficient vitamin D supplementation before and after transplantation; (2) increased 25(OH)D catabolism induced by immunosuppressive drugs (Pascussi *et al.*, 2005; Akeno *et al.*, 2000) and, after renal transplantation, by post-transplant persistent fibroblast growth factor-23 hyper-secretion (Bhan *et al.*, 2006; Pande *et al.*, 2006; Saito *et al.*, 2003); and (3) reduced sun exposure recommended to TR to prevent skin cancers. Serum 25(OH)D levels were analysed in 31 renal TR, who all protected themselves from sun exposure, compared to an age- and gender-matched control group without renal disease or other diseases requiring sun protection at the end of winter. Serum 25(OH)D levels were significantly lower in renal TR compared to controls, with 10 out of 31 having undetectable serum 25(OH)D levels (Querings *et al.*, 2006). It is generally accepted that, due to immunosuppression, TR are at increased risk for UV-induced non-melanoma skin cancers but also for developing malignant melanoma (Reichrath *et al.*, 2010). As a result, TR are now systematically advised to protect themselves from exposure to solar or artificial UV-radiation. However, this represents a serious dilemma, for appr. 80 to 90% of the human body's requirements in vitamin D have to be photosynthesized in the skin from 7-dehydrocholesterol by the action of UVB-radiation. Therefore, due to the lack of solar UV-exposure, careful monitoring of vitamin D status and oral substitution in case of vitamin D insufficiency is of high importance for TR.

29.3 Vitamin D supplementation after transplantation

Despite the high prevalence of vitamin D insufficiency in TR, there is no general consensus regarding vitamin D supplementation after transplantation. It was shown that high doses of vitamin D3 (cholecalciferol; 100,000 IU every other week during 2 months, equivalent to 6,600 IU/day) were able to correct 25(OH)D insufficiency in renal TR, without inducing side effects,

and were associated with a significant decrease in serum PTH concentration. However, this study also indicated that the dose of cholecalciferol used during the maintenance phase (100,000 IU every other month from the sixth to the twelfth month post-transplantation) was insufficient to maintain serum 25(OH)D concentration above 30 ng/ml in half of patients (Courbebaisse *et al.*, 2009). These findings are in consistence with a previous study showing that 25,000 IU of cholecalciferol once a month failed to correct vitamin D insufficiency in renal TR, suggesting that a higher dose of cholecalciferol is necessary to maintain adequate 25(OH)D levels after transplantation (Wissing *et al.*, 2005). The optimal dosage scheme to maintain 25(OH)D concentrations between 30 and 80 ng/ml was simulated from the data of the previous study (Courbebaisse *et al.*, 2009) using a population pharmacokinetic approach. In order to maintain 25(OH)D concentrations between 30-80 ng/ml during the first year after renal transplantation, cholecalciferol dosing should be: six successive administrations of 100,000 IU every two weeks interval, then 100,000 IU once a month until the end of the first year (Benaboud *et al.*, 2012). This theoretical scheme remains to be tested in a prospective study.

After lung, liver and cardiac transplantation, the optimal scheme for vitamin D supplementation remains to be determined.

29.4 Vitamin D and bone loss after transplantation

Improved survival rates after transplantation have been accompanied by increased recognition of previously neglected long-term complications of transplantation such as fractures and osteoporosis due to specific and non-specific risk factors (Bia, 2008). Pre-transplantation bone disease and immunosuppressive therapy, especially corticosteroid, result in rapid bone loss and increased fracture rates early after transplantation (Ebeling, 2007). Bisphosphonates are one of the most promising approaches for the management of transplantation osteoporosis. However, native and active vitamin D metabolites may have additional benefits in reducing hyperparathyroidism, particularly after kidney transplantation. Besides reducing hyperparathyroidism, there are several potential mechanisms by which vitamin D and its analogues may influence post-transplantation bone loss. They may overcome corticosteroid-induced decreases in intestinal calcium absorption and promote differentiation of osteoblast precursors into mature cells (Stein and Shane, 2011). Immediately after renal transplantation, randomized trials showed that active form of vitamin D (Alfacalcidiol) could prevent or attenuate lumbar spine and femoral neck bone loss (El Agroudy *et al.*, 2003, 2005; De Sevaux *et al.*, 2002). Studies of calcitriol have found contradictory results after transplantation, although some report beneficial effects at doses >0.5 μg/day after (Stein and Shane, 2011). Most studies using active forms of vitamin D were not powered to assess difference in fracture rates.

Concerning native vitamin D, it was shown in paediatric renal TR that low serum 25(OH)D correlates with hyperparathyroidism and with short stature (Shroff *et al.*, 2011). In adult renal TR, efficient treatment of vitamin D insufficiency with cholecalciferol was shown to reduce serum PTH concentration (Courbebaisse *et al.*, 2009). In two studies, native vitamin D, at doses of 800

IU daily in 40 patients (Al Gabri *et al.*, 2005) or 25,000 IU monthly in 90 patients (Wissing *et al.*, 2005), did not prevent bone loss after kidney transplantation. However, randomized studies remain to be performed to assess the potential effect of higher doses of native vitamin D on bone mineral density and fracture after transplantation.

29.5 Vitamin D and *de novo* type 2 diabetes mellitus after transplantation

According to the diagnostic criteria and post-transplantation delay, *de novo* type 2 DM occurs in 10% to 30% of renal TR, mainly due to corticosteroid and tacrolimus treatment (Montori *et al.*, 2002).

The potential effects of vitamin D on insulin secretion and insulin resistance are supported by many experimental data (Pittas *et al.*, 2007b). First, VDR (Johnson *et al.*, 1994) and CYP27B1 (Bland *et al.*, 2004) are expressed in pancreatic β cells, and VDRE have been identified in the promoter of human gene encoding for insulin (Maestro *et al.*, 2003). Second, *in vitro* studies showed that calcitriol stimulates insulin human gene transcription and expression of insulin receptor and glucose transport (Bourlon *et al.*, 1999; Norman *et al.*, 1980; Kadowaki and Norman, 1984; Tanaka *et al.*, 1984). Third, VDR KO mice have an altered insulin secretion (Zeitz *et al.*, 2003), and vitamin D3 supplementation increases glucose tolerance and insulin secretion in vitamin D deficient rats (Cade and Norman, 1986). In humans, serum 25(OH)D concentrations are inversely correlated to type 2 DM prevalence (Pittas *et al.*, 2007b; Scragg *et al.*, 2004). Vitamin D insufficiency is also associated with increased HbA_{1c} levels (Hypponen and Power, 2006) and resistance to insulin (Scragg *et al.*, 2004). Recent data suggest that the influence of vitamin D on the resistance to insulin may be partly mediated by a control of adiponectin gene by calcitriol (Sun and Zemel, 2007).

Few interventional studies reported positive effect of vitamin D supplementation on intermediary parameters of insulin resistance. A double-blind placebo randomized trial indicated that vitamin D3 supplementation improves postprandial insulin sensitivity in apparently healthy men likely to have insulin resistance (centrally obese but non-diabetic) (Nagpal *et al.*, 2009). In another double-blind placebo randomized trial, supplementation with oral calcium (500 mg per day) and vitamin D3 (700 IU per day) during 3 years improved fasting glycaemia in hyperglycaemic subjects (Pittas *et al.*, 2007a). More recently, it was shown showed that supplementation with vitamin D3 (2000 IU per day during 4 months *versus* placebo) could improve pancreatic β cell function in patients at high risk of type 2 DM (Mitri *et al.*, 2011). However, other studies aiming at testing the effect of various native vitamin D formulations on different parameters of type 2 DM, including the women's health initiative one (De Boer *et al.*, 2008), showed no effect of vitamin D supplementation.

No study reported the potential effect of activated or native vitamin D on *de novo* type 2 DM after transplantation.

29.6 Vitamin D and cardiovascular risk after transplantation

In comparison with the general population, TR have an increased cardiovascular risk both secondary to traditional and non-traditional risk factors (50-fold higher in renal TR) (Sarnak *et al.*, 2003). Several observational and experimental data argue in favour of a potential protective role of vitamin D against cardiovascular diseases. Cross-sectional studies indicated that vitamin D deficiency might be associated with arteriosclerosis and endothelial dysfunction in end-stage renal disease patients (London *et al.*, 2007). Several prospective case-control studies have reported a strong association between low circulating levels of 25(OH)D and an increased risk of major cardiovascular events such as myocardial infarction, stroke, congestive heart failure (Wang *et al.*, 2008; Giovannucci *et al.*, 2008), and cardiovascular disease death (Pilz *et al.*, 2008, 2009; Dobnig *et al.*, 2008). These associations remained significant after adjustment for other risk factors of cardiovascular disease.

Possible explanations for these findings involve both direct and indirect effects of vitamin D on cardiovascular function. Direct effects are supported by the fact that cardiomyocytes, vascular smooth muscle cells, and endothelial cells express both the VDR and the CYP27B1 enzyme (Zittermann *et al.*, 2005). Furthermore, genes up-regulated during myocardial hypertrophy (e.g. atrial natriuretic peptide) possess VDRE and are suppressed by calcitriol in animal or cell models (Wu *et al.*, 1996). Similarly, in cultured cells, calcitriol inhibits cardiomyocytes proliferation (O'Connell *et al.*, 1997; Nibbelink *et al.*, 2007) and stimulates vascular smooth muscle cells proliferation and vascular endothelial growth factor expression by these cells (Cardus *et al.*, 2006). Calcitriol also modulates contractile performances of isolated rat or mouse cardiomyocytes (Green *et al.*, 2006; Tishkoff *et al.*, 2008). Potential indirect effects of vitamin D concern several risk factors for cardiovascular dysfunction. Regarding RAS, VDR and CYP27B1 KO mice have high levels of blood pressure and cardiac hypertrophy due to increased activation of RAS (Bouillon *et al.*, 2008), and calcitriol treatment inhibits renin activation and decreases blood pressure and cardiac hypertrophy in CYP27B1 KO mice (Qiao *et al.*, 2005; Sigmund, 2002; Zhou *et al.*, 2008). Regarding MMP, calcitriol reduces the expression of MMP2 and MMP9 (Nakagawa *et al.*, 2005), two MMP that may promote vascular calcification (Basalyga *et al.*, 2004).

Even though prospective clinical studies are currently lacking, numerous studies have shown positive effects of vitamin D supplementation on intermediary parameters potentially related to cardiovascular health. A placebo-controlled trial showed an improvement in the pro-inflammatory and anti-inflammatory cytokine profile of patients with congestive heart failure (Schleithoff *et al.*, 2006) and an improvement in endothelial function in vitamin D deficient subjects receiving native vitamin D (Tarcin *et al.*, 2009). A recent meta-analysis of 11 randomized trials either with activated or native vitamin D confirmed this reduction in systolic blood pressure in hypertensive patients and suggested that vitamin D produced a greater fall in systolic blood pressure than activated compounds (Witham *et al.*, 2009). However, because vitamin D intoxication in humans may lead to vascular calcifications, the benefit or risk ratio of supra-physiologic dosages of vitamin D should be evaluated, especially in TR having an increased

cardiovascular risk, since a biphasic effect of vitamin D on the risk of vascular calcifications may exist (Zittermann *et al.*, 2007).

29.7 Vitamin D and cancer risk after transplantation

TR experience an increased incidence of cancers, especially for non-melanoma skin cancers and non-Hodgkin lymphoma. Three years after renal transplantation, US Renal Data System has shown a 7.5% cumulative incidence of non-skin cancers and a 7.4% cumulative incidence of skin cancers in renal TR (Kasiske *et al.*, 2004).

Several environmental studies have reported that living at higher latitudes increased the risk of developing colon, prostate, breast, lymphoma, and several other cancers (Garland and Garland, 2006), which may be linked to decreased vitamin D3 synthesis. Many prospective case-control studies have showed that adults in the highest quantile of 25(OH)D levels have a 30% to 50% decreased risk of colon (Giovannucci, 2007), breast (Garland *et al.*, 2007), prostate (Ahonen *et al.*, 2000), and ovary (Tworoger *et al.*, 2007) cancers compared with those in the lowest quantile. Furthermore, the risk of non-Hodgkin lymphoma is 30% to 40% lower in adults with high vitamin D intakes or great sun exposure (Soni *et al.*, 2007; Polesel *et al.*, 2006), and retrospective studies suggest an association between low serum 25(OH)D level and death from cancer (Tretli *et al.*, 2009; Fedirko *et al.*, 2012; Ren *et al.*, 2012*).*

Furthermore, the role of poor vitamin D status and cancer risk has received strong experimental support from the consistent demonstration that activation of the VDR by calcitriol produced locally induces differentiation (Liu *et al.*, 1996) and apoptosis (Diaz *et al.*, 2000) and inhibits proliferation (Tangpricha *et al.*, 2001) and angiogenesis (Mantell *et al.*, 2000). Moreover, vitamin D and its metabolites stimulate mutual adherence of cells and intercellular communication through gap junctions, thereby decreasing metastatic potential and strengthening the inhibition of proliferation that results from intercellular tight physical contact (Fleet, 2008).

In humans, the data are still controversial. In the women's health initiative study, 36,282 women were randomized to receive either a placebo or 1000 mg calcium and 400 IU vitamin D3 daily. Although a strong negative relationship between baseline 25(OH)D levels and the incidence of colorectal cancer was found, no reduction in the incidence of cancers was observed in the treated group compared with the placebo group (Wactawski-Wende *et al.*, 2006). However in this study, adherence to treatment was poor and cholecalciferol dosage was considered to be too low by many experts. More recently, a 4-year double blind randomized placebo-controlled trial including 1,180 postmenopausal women showed a significant decreased risk of cancers in the group receiving calcium (1,500 mg/day) and vitamin D3 (1,100 IU vitamin D3/day) supplementation (Lappe *et al.*, 2007).

The potential protective role of vitamin D against cancer risk in the general population was also assessed in renal TR (Ducloux *et al.*, 2008). In a cohort of 363 renal TR followed up during 3 to 5

years after transplantation, a higher incidence of post-transplant cancers was observed in patients with pre-transplant 25(OH)D concentrations less than 10 ng/ml (13.7% vs. 3.7% for those with 25(OH)D levels >30 ng/ml, P=0.007). This result remains to be confirmed thanks to interventional randomized trials. However, caution should be considered when increasing vitamin D dosage as a few observational retrospective studies suggest that high 25(OH)D values may be deleterious for the risk of prostate cancer (Tuohimaa *et al.*, 2004). An intriguing question is the relationship between sun exposure and non-melanoma skin cancer. Even though the role of sun exposure has been emphasized, one should note that VDR KO mice develop UVB-induced skin cancers more rapidly and more frequently than wild-type mice, suggesting a potential protective role for 25(OH)D against non-melanoma skin cancers (Ellison *et al.*, 2008).

29.8 Vitamin D and infections after transplantation

The newer potent immunosuppressive drugs have decreased the incidence of rejection in TR while increasing susceptibility to opportunistic infections. Therefore, even though the patterns of infections after transplantation have been altered by routine antimicrobial prophylaxis, these complications remain an important issue in this population (Fishman, 2007).

Recent retrospective data from hemodialysis patients suggest that regular vitamin D dosing did not show benefit for infectious mortality (St Peter *et al.*, 2009). However, in humans, the risk of being infected with MT and the risk of developing upper respiratory tract infections have been shown to be associated with vitamin D deficiency (Cannell *et al.*, 2006). Experimental data support this possible effect. First, it is recognized that macrophages express both VDR and CYP27B1 (Mora *et al.*, 2008). Second, it has been shown that infection of macrophages with MT induced a toll-like receptors response with a signal transduction to the nucleus to increase VDR and CYP27B1 expression. Calcitriol production by macrophages and calcitriol action on macrophages stimulate expression of cathelicidin, an antimicrobial peptide, by these cells. Cathelicidin in turn can kill infective agents, such as MT, within the macrophage (Liu *et al.*, 2006). Finally, calcitriol also stimulates human monocyte proliferation *in vitro* (Ohta *et al.*, 1985) and increases IL-1 production by monocytes and macrophages (Bhalla *et al.*, 1986).

A placebo-controlled trial demonstrated a reduction in influenza infections in patients treated with 2,000 IU vitamin D3/day compared with the placebo group (Aloia and Li-Ng, 2007). More recently, it was reported that vitamin D3 supplementation could improve viral response in hepatitis C virus infected patients receiving the classical interferon/ribavirin treatment (Abu-Mouch *et al.*, 2011), and was also able to hasten sputum culture conversion in adults infected with MT with the tt genotype of the TaqI VDR polymorphism (Martineau *et al.*, 2011).

Although low serum 25(OH)D levels in lung TR were associated with increased incidence of infection (Lowery *et al.*, 2012), the potential protective role of vitamin D against infections after transplantation remains to be proven.

29.9 Vitamin D and graft rejection

Even though greatly reduced, AR and chronic allograft deterioration remain important issues after transplantation.

In retrospective case-control studies, it was reported that osteoporotic renal TR experienced fewer AR after calcitriol treatment induction (Tanaci *et al.*, 2003) and needed fewer pulse steroid doses (Sezer *et al.*, 2005). In a retrospective study after liver transplantation, early vitamin D3 supplementation was independently associated with a lack of AR (Bitetto *et al.*, 2010) and low 25(OH)D levels were associated with increased incidence of AR in lung TR (Lowery *et al.*, 2012).

Experimental studies also support this effect. First, calcitriol inhibits the expression of IL-2 and interferon-γ mRNA and protein in T cells and attenuates CD4+ and CD8+ T-cell proliferation and their cytotoxic activity (Lemire *et al.*, 1985; Reichel *et al.*, 1987; Rigby *et al.*, 1987). VDR expression in T cells is not constitutive but only up-regulated after activation (Veldman *et al.*, 2000). Calcitriol also decreases plasma cell differentiation, B-cell proliferation, and IgG secretion (Chen *et al.*, 2007). Second, calcitriol inhibits the expression of major histocompatibility complex class II, CD40, CD80, and CD86, thus decreasing differentiation, maturation, and immunostimulatory capacity of DCs (Fritsche *et al.*, 2003; Penna and Adorini, 2000; Griffin *et al.*, 2001), which constitutively express VDR and CYP27B1 (Fritsche *et al.*, 2003). Even though DCs are not the most important cells implicated with the alloimmune response, their role has been recently emphasized (Chen, 2005). Calcitriol also down-regulates IL-12 synthesis and simultaneously stimulates IL-10 production by DCs (Penna and Adorini, 2000). The net result is a decrease in T helper 1-cell responses and a possible induction of IL-10 producing T-regulatory type 1 cells. By limiting adaptative immune response and down-regulating DCs proliferation and activity, VDR agonists are, thus, susceptible to help in preventing AR (Mora *et al.*, 2008). The potential protective role of VDR agonists against chronic allograft rejection is supported by many arguments. In addition to potentially inducing tolerogenic DCs, VDR agonists could also inhibit the production of chemokines, responsible for leukocytes infiltration in vessels allograft, and may down-regulate TGF-β pathway, which has a profibrotic activity (Adorini *et al.*, 2005). Other renoprotective effects of vitamin D may participate in the prevention of chronic allograft rejection (see the following paragraph). The immunomodulatory capacity of calcitriol observed *in vitro* has been confirmed in several animal models of transplantation. Calcitriol or its analogs prolonged the survival of murine cardiac allograft (Lemire *et al.*, 1992), increased survival in a rat model of liver transplantation (Zhang *et al.*, 2003), and delayed autoimmune disease recurrence after syngeneic islet transplantation in nonobese diabetic mice (Casteels *et al.*, 1998). In addition, active compounds of vitamin D were shown to protect from chronic allograft rejection in a rat model of renal transplantation (Hullett *et al.*, 2005) and in a mouse model of aortic transplantation (Amuchastegui *et al.*, 2005). Importantly, Cantorna *et al.* (1998) showed that calcitriol prolonged graft survival in animal models without increasing the risk of fungal or viral infection.

In a small prospective study, Ardalan *et al.* (2007) investigated the effect of calcitriol therapy which was started in the donor and continued in recipient side (9 donors, who all received

calcitriol at a dose of 0.5 μg/day orally for 5 days before donation and 9 TR who were treated with the same regimen for one month post-transplant and thereafter received 0.25 μg/day for five months compared to 10 controls with conventional treatment). Although only 19 renal TR were included, a significant expansion of CD4+ CD25+ Tregs in the calcitriol-treated group could be demonstrated, providing strong evidence of the immunomodulatory properties of active vitamin D after transplantation (Ardalan *et al.*, 2007). In another small prospective clinical trial, 24 patients transplanted 6-18 months before the study were treated with calcitriol at a dose of 0.5 μg/day during four weeks. A decrease in HLA-DR, CD28, CD86, and CD40 expression on white blood cells was found after calcitriol treatment (Ahmadpoor *et al.*, 2009).

Altogether, VDR agonists could be used as potentially immunomodulatory agents in TR. Currently, calcitriol analogs such as paricalcitol, exerting immunomodulation without inducing hypercalcemia, are being developed (Van Etten and Mathieu, 2005).

29.10 Renoprotective effects of vitamin D after transplantation

A number of observational clinical studies have confirmed the potent renoprotective and antiproteinuric effect of vitamin D and its analogs (Agarwal *et al.*, 2005; Li, 2009). Importantly, 25(OH)D level was also found to be inversely associated with prevalence of albuminuria (De Boer *et al.*, 2007) and to be an independent inverse predictor of progression to dialysis in patients with stage 2 to 5 chronic kidney disease (Ravani *et al.*, 2009). After renal transplantation, vitamin D insufficiency was significantly associated with proteinuria (Lee *et al.*, 2011) and another retrospective study suggested a beneficial effect of calcitriol therapy on renal graft function (O'Herrin *et al.*, 2002).

Experimental data support these observations. As mentioned earlier, calcitriol and analogs inhibit RAS, which has a pivotal role in glomerular and tubulo-interstitial damages, glomerular hypertension, and proteinuria (Brewster and Perazella, 2004). It has also been shown that vitamin D inhibits NF-κB activation (Sun *et al.*, 2006), which is known to play an important role in renal diseases by promoting inflammation and fibrogenesis (Guijarro and Egido, 2001). In many animal models of chronic kidney disease, evidence for the renoprotective effects of vitamin D analogs were shown because of the attenuation of tubulo-interstitial fibrosis and glomerulosclerosis, reduced proteinuria, repression of extracellular matrix production, EMT markers, and TGF-β pathway (Kuhlmann *et al.*, 2004; Schwarz *et al.*, 1998; Tan *et al.*, 2006). These effects were independent of PTH (Schwarz *et al.*, 1998; Kuhlmann *et al.*, 2004) and at least in part dependent of RAS inhibition (Freundlich *et al.*, 2008). Of interest, losartan or paricalcitol alone moderately improved kidney injury in a model of diabetic mice, whereas the combination of both molecules prevented albuminuria and reduced glomerulosclerosis (Zhang *et al.*, 2008). Despite its benefits as a potent immunosuppressive agent, the use of cyclosporine A is limited by its nephrotoxic properties after transplantation. Interestingly, in a rat model, paricalcitol appears to attenuate cyclosporine A-induced nephropathy by suppression of inflammatory, pro-fibrotic,

and apoptotic factors through inhibition of the NF-κB, Smad, and mitogen-activated protein kinase signaling pathways (Park *et al.*, 2010).

In humans, a recent randomized placebo-controlled, double-blind trial showed that addition of 2 μg/day paricalcitol to RAS inhibition safely lowers residual albuminuria in patients with diabetic nephropathy (De Zeeuw *et al.*, 2010).

Very few studies investigated the potential nephroprotective role of vitamin D after transplantation. In a recent study, glomerular filtration rates using iohexol plasma clearance, urinary procollagen III aminoterminal propeptide excretion, epithelial phenotypic changes as markers of EMT and Banff scores at 3 and 12 months after renal transplantation were analysed in 64 renal TR with or without cholecalciferol supplementation between months 3 and 12. The scheme of cholecalciferol treatment used in the treated group was described previously (Courbebaisse *et al.*, 2009). In the treated group, cholecalciferol supplementation did not prevent EMT, interstitial fibrosis, tubular atrophy, or renal function deterioration. These results challenged the experimental data, suggesting that vitamin D-analog supplementation confers nephroprotection. However, these negative results should be interpreted with caution because of the limited follow-up, the small size of the population and the lack of efficiency of the maintenance treatment to maintain serum 25(OH)D above 30 ng/ml in the treated group (Courbebaisse *et al.*, 2011).

29.11 Vitamin D and mortality after transplantation

In a meta-analysis including 18 independent randomized controlled trials, intake of ordinary doses of vitamin D supplements (mean daily dose=528 IU) seemed to be associated with decrease in total mortality from any cause (Autier and Gandini, 2007). In hemodialysis patients (Naves-Diaz *et al.*, 2008; Teng *et al.*, 2005; Tentori *et al.*, 2006), and in patients with chronic kidney disease (Kovesdy *et al.*, 2008; Shoben *et al.*, 2008), treatment with calcitriol was associated with significantly higher survival. Level of 25(OH)D was also shown to be an independent inverse predictor of death in patients with stage 2 to 5 chronic kidney disease (Ravani *et al.*, 2009). Interestingly, hemodialysis patients receiving paricalcitol had a survival advantage over those treated with calcitriol (Teng *et al.*, 2003). Whether these results may apply to TR is unknown although low post-operative calcitriol concentrations were independently associated with 1-year mortality in heart TR (Zittermann *et al.*, 2009). Moreover, the mortality of lung TR who remained vitamin D deficient 1 year post-transplant was higher than that of recipients who maintained normal vitamin D level at 1 year post-transplant (Lowery *et al.*, 2012).

29.12 Conclusion and perspectives

In addition to classical effects on bone and mineral metabolism, vitamin D displays a wide spectrum of non-classical effects that, for most, are especially relevant for the care of TR. These pleiotropic effects are mostly documented by observational and experimental studies or small

intervention trials that most often evaluated intermediate parameters. The time has now come for large placebo-controlled trials in TR, using larger dosages of vitamin D than the current recommended intakes and targeting clinical endpoints. Development of calcitriol analogs with minimal calcemic effects may allow taking advantage of the pleiotropic effects of vitamin D with fewer side effects and is a 'hot' research topic.

Two randomized control studies are actually undergoing to test the effect of cholecalciferol after renal transplantation:

- The objective of the VITA-D study (Vitamin D3 Substitution in Vitamin D Deficient Kidney Transplant Recipients; ClinicalTrials.gov Identifier: NCT00752401) is to conduct a randomized, double-blind, placebo-controlled study focusing on the impact of cholecalciferol substitution in vitamin D deficient renal TR on graft function (MDRD estimated GFR), incidence of acute rejection episodes, post-transplant infections within the first year after transplantation. Two hundred renal TR with 25(OH)D <20 ng/ml at time of transplantation will be randomized to receive either cholecalciferol (6,800 IU/day during one year) or placebo (Thiem *et al.*, 2009).
- The VITALE study (VITamine D Supplementation in RenAL Transplant Recipients; ClinicalTrials.gov Identifier: NCT01431430) is a randomized, controlled, double-blind study whose objective is to evaluate the influence of vitamin D3 substitution in vitamin D deficient (25(OH)D <30 ng/ml) renal TR on the post-transplant outcome. The primary purpose of this study is to compare the effects of high dose (100,000 IU forthightly for 2 months then monthly for 22 months) *versus* low dose (12,000 IU forthightly for 2 months then monthly for 22 months) of cholecalciferol on a composite endpoint consisting in *de novo* DM and cancers, cardiovascular diseases, and patient death. 640 TR between 12 and 48 months after transplantation will be randomized to blindly receive either high or low dose of cholecalciferol with a follow-up of 2 years.

References

Abu-Mouch, S., Fireman, Z., Jarchovsky, J., Zeina, A.R. and Assy, N., 2011. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. World J Gastroenterol 17, 5184-5190.

Adorini, L., Amuchastegui, S. and Daniel, K.C., 2005. Prevention of chronic allograft rejection by vitamin D receptor agonists. Immunol Lett 100, 34-41.

Agarwal, R., Acharya, M., Tian, J., Hippensteel, R.L., Melnick, J.Z., Qiu, P., Williams, L. and Batlle, D., 2005. Antiproteinuric effect of oral paricalcitol in chronic kidney disease. Kidney Int 68, 2823-2828.

Ahmadpoor, P., Ilkhanizadeh, B., Ghasemmahdi, L., Makhdoomi, K. and Ghafari, A., 2009. Effect of active vitamin D on expression of co-stimulatory molecules and HLA-DR in renal transplant recipients. Exp. Clin. Transplant. 7, 99-103.

Ahonen, M.H., Tenkanen, L., Teppo, L., Hakama, M. and Tuohimaa, P., 2000. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control 11, 847-852.

Akeno, N., Matsunuma, A., Maeda, T., Kawane, T. and Horiuchi, N., 2000. Regulation of vitamin D-1alpha-hydroxylase and -24-hydroxylase expression by dexamethasone in mouse kidney. J Endocrinol 164, 339-348.

Al-Gabri, S., Zadrazil, J., Krejci, K., Horak, P. and Bachleda, P., 2005. Changes in bone mineral density and selected metabolic parameters over 24 months following renal transplantation. Transplant Proc 37, 1014-1019.

Aloia, J.F. and Li-Ng, M., 2007. Re: Epidemic influenza and vitamin D. Epidemiol Infect 135, 1095-1096.

Amuchastegui, S., Daniel, K.C. and Adorini, L., 2005. Inhibition of acute and chronic allograft rejection in mouse models by BXL-628, a nonhypercalcemic vitamin D receptor agonist. Transplantation 80, 81-87.

Ardalan, M.R., Maljaei, H., Shoja, M.M., Piri, A.R., Khosroshahi, H.T., Noshad, H. and Argani, H., 2007. Calcitriol started in the donor, expands the population of CD4+CD25+ T cells in renal transplant recipients. Transplant. Proc 39, 951-953.

Autier, P. and Gandini, S., 2007. Vitamin D supplementation and total mortality: A meta-analysis of randomized controlled trials. Arch Intern Med 167, 1730-1737.

Basalyga, D.M., Simionescu, D.T., Xiong, W., Baxter, B.T., Starcher, B.C. and Vyavahare, N.R., 2004. Elastin degradation and calcification in an abdominal aorta injury model: role of matrix metalloproteinases. Circulation 110, 3480-3487.

Benaboud, S., Urien, S., Thervet, E., Prié, D., Legendre, C., Souberbielle, J.C., Hirt, D., Friedlander, G., Treluyer, J.M. and Courbebaisse, M, 2012. Determination of optimal cholecalciferol treatment in renal transplant recipients using a population pharmacokinetic approach. European Journal of Clinical Pharmacology, in press. DOI: http://dx.doi.org/10.1007/s00228-012-1378-3.

Bhalla, A.K., Amento, E.P. and Krane, S.M., 1986. Differential effects of 1, 25-dihydroxyvitamin D3 on human lymphocytes and monocyte/macrophages: inhibition of interleukin-2 and augmentation of interleukin-1 production. Cell Immunol 98, 311-322.

Bhan, I., Shah, A., Holmes, J., Isakova, T., Gutierrez, O., Burnett, S.M., Juppner, H. and Wolf, M., 2006. Post-transplant hypophosphatemia: tertiary 'hyper-phosphatoninism'? Kidney Int 70, 1486-1494.

Bia, M., 2008. Evaluation and management of bone disease and fractures post transplant. Transplant Rev (Orlando) 22, 52-61.

Bischoff-Ferrari, H.A., Giovannucci, E., Willett, W.C., Dietrich, T. and Dawson-Hughes, B., 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 84, 18-28.

Bland, R., Markovic, D., Hills, C.E., Hughes, S.V., Chan, S.L., Squires, P.E. and Hewison, M., 2004. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. J Steroid Biochem Mol Biol 90, 121-125.

Bouillon, R., Carmeliet, G., Verlinden, L., Van Etten, E., Verstuyf, A., Luderer, H.F., Lieben, L., Mathieu, C. and Demay, M., 2008. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 29, 726-776.

Bourlon, P.M., Billaudel, B. and Faure-Dussert, A., 1999. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on *de novo* insulin biosynthesis in the islets of the rat endocrine pancreas. J Endocrinol 160, 87-95.

Brewster, U.C. and Perazella, M.A., 2004. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. Am J Med 116, 263-272.

Cade, C. and Norman, A.W., 1986. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat *in vivo*. Endocrinology 119, 84-90.

Cannell, J.J., Vieth, R., Umhau, J.C., Holick, M.F., Grant, W.B., Madronich, S., Garland, C.F. and Giovannucci, E., 2006. Epidemic influenza and vitamin D. Epidemiol Infect 134, 1129-1140.

Cantorna, M.T., Hullett, D.A., Redaelli, C., Brandt, C.R., Humpal-Winter, J., Sollinger, H.W. and De Luca, H.F., 1998. 1,25-Dihydroxyvitamin D3 prolongs graft survival without compromising host resistance to infection or bone mineral density. Transplantation 66, 828-831.

Cardús, A., Parisi, E., Gallego, C., Aldea, M., Fernández, E. and Valdivielso, J.M., 2006. 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. Kidney Int 69, 1377-1384.

Casteels, K., Waer, M., Laureys, J., Valckx, D., Depovere, J., Bouillon, R. and Mathieu, C., 1998. Prevention of autoimmune destruction of syngeneic islet grafts in spontaneously diabetic nonobese diabetic mice by a combination of a vitamin D3 analog and cyclosporine. Transplantation 65, 1225-1232.

Chen, S., Sims, G.P., Chen, X.X., Gu, Y.Y., Chen, S. and Lipsky, P.E., 2007. Modulatory effects of 1, 25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol 179, 1634-1647.

Chen, W., 2005. The role of plasmacytoid dendritic cells in immunity and tolerance. Curr Opin Organ Transplant 10, 181.

Courbebaisse, M., Xu-Dubois, Y.C., Thervet, E., Prié, D., Zuber, J., Kreis, H., Legendre, C., Rondeau, E. and Pallet, N., 2011. Cholecalciferol supplementation does not protect against renal allograft structural and functional deterioration: a retrospective study.Transplantation 91, 207-212.

Courbebaisse, M., Thervet, E., Souberbielle, J.C., Zuber, J., Eladari, D., Martinez, F., Mamzer-Bruneel, M.F., Urena, P., Legendre, C., Friedlander, G. and Prie, D., 2009. Effects of vitamin D supplementation on the calcium-phosphate balance in renal transplant patients. Kidney Int 75, 646-651.

Bitetto, D., Fabris, C., Falleti, E., Fornasiere, E., Fumolo, E., Fontanini, E., Cussigh, A., Occhino, G., Baccarani, U., Pirisi, M. and Toniutto, P., 2010. Vitamin D and the risk of acute allograft rejection following human liver transplantation. Liver Int 30, 417-444.

Dawson-Hughes, B., Heaney, R.P., Holick, M.F., Lips, P., Meunier, P.J. and Vieth, R., 2005. Estimates of optimal vitamin D status. Osteoporos Int 16, 713-716.

De Boer, I.H., Tinker, L.F., Connelly, S., Curb, J.D., Howard, B.V., Kestenbaum, B., Larson, J.C., Manson, J.E., Margolis, K.L., Siscovick, D.S., Weiss, N.S. and the Women's Health Initiative Investigators, 2008. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 31, 701-707.

De Boer, I.H., Ioannou, G.N., Kestenbaum, B., Brunzell, J.D. and Weiss, N.S., 2007. 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). Am J Kidney Dis 50, 69-77.

De Sevaux, R.G., Hoitsma, A.J., Corstens, F.H. and Wetzels, J.F., 2002. Treatment with vitamin D and calcium reduces bone loss after renal transplantation: a randomized study. J. Am. Soc. Nephrol 13, 1608-1614.

De Zeeuw, D., Agarwal, R., Amdahl, M., Audhya, P., Coyne, D., Garimella, T., Parving, H.H., Pritchett, Y., Remuzzi, G., Ritz, E. and Andress, D., 2010. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet 376, 1543-1551.

Díaz, G.D., Paraskeva, C., Thomas, M.G., Binderup, L. and Hague, A., 2000. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: Possible implications for prevention and therapy. Cancer Res 60, 2304-2312.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., Kinkeldei, J., Boehm, B.O., Weihrauch, G. and Maerz, W., 2008. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Arch Intern Med 168, 1340-1349.

Ducloux, D., Courivaud, C., Bamoulid, J., Kazory, A., Dumoulin, G. and Chalopin, J.M., 2008. Pretransplant serum vitamin D levels and risk of cancer after renal transplantation. Transplantation 85, 1755-1759.

Ebeling, P.R., 2007. Transplantation osteoporosis. Curr Osteoporos Rep 5, 29-37.

El-Agroudy, A.E., El-Husseini, A.A., El-Sayed, M., Mohsen, T. and Ghoneim, M.A., 2005. A prospective randomized study for prevention of postrenal transplantation bone loss. Kidney Int 67, 2039-2045.

El-Agroudy, A.E., El-Husseini, A.A., El-Sayed, M. and Ghoneim, M.A., 2003. Preventing bone loss in renal transplant recipients with vitamin D. J Am Soc Nephrol 14, 2975-2979.

Ellison, T.I., Smith, M.K., Gilliam, A.C. and MacDonald, P.N., 2008. Inactivation of the vitamin D receptor enhances susceptibility of murine skin to UV-induced tumorigenesis. J Invest Dermatol 128, 2508-2517.

Fedirko, V., Riboli, E., Tjønneland, A., Ferrari, P., Olsen, A., Bueno-de-Mesquita, H.B., Van Duijnhoven, F.J., Norat, T., Jansen, E.H., Dahm, C.C., Overvad, K., Boutron-Ruault, M.C., Clavel-Chapelon, F., Racine, A., Lukanova, A., Teucher, B., Boeing, H., Aleksandrova, K., Trichopoulou, A., Benetou, V., Trichopoulos, D., Grioni, S., Vineis, P., Panico, S., Palli, D., Tumino, R., Siersema, P.D., Peeters, P.H., Skeie, G., Brustad, M., Chirlaque, M.D., Barricarte, A., Ramón Quirós, J., Sánchez, M.J., Dorronsoro, M., Bonet, C., Palmqvist, R., Hallmans, G., Key, T.J., Crowe, F., Khaw, K.T., Wareham, N., Romieu, I., McKay, J., Wark, P.A., Romaguera, D. and Jenab, M., 2012. Prediagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms, and survival in patients with colorectal cancer in western European ppulations. Cancer Epidemiol Biomarkers Prev 21, 582-593.

Fishman, J.A., 2007. Infection in solid-organ transplant recipients. N Engl J Med; 357: 2601-2614.

Fleet, J.C., 2008. Molecular actions of vitamin D contributing to cancer prevention. Mol Aspects Med 29, 388-396.

Freundlich, M., Quiroz, Y., Zhang, Z., Zhang, Y., Bravo, Y., Weisinger, J.R., Li, Y.C. and Rodriguez-Iturbe, B., 2008. Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. Kidney Int 74, 1394-1402.

Fritsche, J., Mondal, K., Ehrnsperger, A., Andreesen, R. and Kreutz, M., 2003. Regulation of 25-hydroxyvitamin D3–1 alpha-hydroxylase and production of 1 alpha,25-dihydroxyvitamin D3 by human dendritic cells. Blood 102, 3314-3316.

Garland, C.F. and Garland, F.C., 2006. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol 35, 217-220.

Garland, C.F., Gorham, E.D., Mohr, S.B., Grant, W.B., Giovannucci, E.L., Lipkin, M., Newmark, H., Holick, M.F. and Garland, F.C., 2007. Vitamin D and prevention of breast cancer: Pooled analysis. J Steroid Biochem Mol Biol 103, 708-711.

Giovannucci, E., 2007. Epidemiological evidence for vitamin D and colorectal cancer. J Bone Miner Res 22 (suppl 2), V81-V85.

Giovannucci, E., Liu, Y., Hollis, B.W. and Rimm, E.B., 2008. 25-hydroxyvitamin D and risk of myocardial infarction in men: A prospective study. Arch Intern Med 168, 1174-1180.

Green, J.J., Robinson, D.A., Wilson, G.E., Simpson, R.U. and Westfall, M.V., 2006. Calcitriol modulation of cardiac contractile performance via protein kinase C. J Mol Cell Cardiol 41, 350-359.

Griffin, M.D., Lutz, W., Phan, V.A., Bachman, L.A., McKean, D.J. and Kumar, R., 2001. Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 and its analogs: A vitamin D receptor-dependent pathway that promotes a persistent state of immaturity *in vitro* and *in vivo*. Proc Natl Acad Sci USA 98, 6800-6805.

Guijarro, C. and Egido, J., 2001. Transcription factor-kappa B (NF-kappa B) and renal disease. Kidney Int 59, 415-424.

Heaney, R.P., 2003. Vitamin D depletion and effective calcium absorption. J Bone Miner Res 18, 1342; author reply 1343.

Holick, M.F., 2007. Vitamin D deficiency. N Engl J Med 357, 266-281.

Hullett, D.A., Laeseke, P.F., Malin, G., Nessel, R., Sollinger, H.W. and Becker, B.N., 2005. Prevention of chronic allograft nephropathy with vitamin D. Transpl Int 18, 1175-1186.

Hypponen, E. and Power, C., 2006. Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity. Diabetes Care 29, 2244-2246.

Johnson, J.A., Grande, J.P., Roche, P.C. and Kumar, R., 1994. Immunohistochemical localization of the 1,25(OH)2D3 receptor and calbindin D28k in human and rat pancreas. Am J Physiol, 267, E356-E360.

Kadowaki, S. and Norman, A.W., 1984. Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. J Clin Invest 73, 759-766.

Kasiske, B.L., Snyder, J.J., Gilbertson, D.T. and Wang, C., 2004. Cancer after kidney transplantation in the United States. Am J Transplant 4, 905-913.

Kovesdy, C.P., Ahmadzadeh, S., Anderson, J.E. and Kalantar-Zadeh, K., 2008. Association of activated vitamin D treatment and mortality in chronic kidney disease. Arch Intern Med 168, 397-403.

Kuhlmann, A., Haas, C.S., Gross, M.L., Reulbach, U., Holzinger, M., Schwarz, U., Ritz, E. and Amann, K., 2004. 1,25-Dihydroxyvitamin D3 decreases podocyte loss and podocyte hypertrophy in the subtotally nephrectomized rat. Am J Physiol Renal Physiol 286, F526-F533.

Lappe, J.M., Travers-Gustafson, D., Davies, K.M., Recker, R.R. and Heaney, R.P., 2007. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 85, 1586-1591.

Lee, D.R., Kong, J.M., Cho, K.I. and Chan, L., 2011.Impact of vitamin D on proteinuria, insulin resistance, and cardiovascular parameters in kidney transplant recipients. Transplant Proc 43, 3723-3729.

Lemire, J.M., Adams, J.S., Kermani-Arab, V., Bakke, A.C., Sakai, R. and Jordan, S.C., 1985. 1,25-dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity *in vitro*. J Immunol 134, 3032-3035.

Lemire, J.M., Archer, D.C., Khulkarni, A., Ince, A., Uskokovic, M.R. and Stepkowski, S., 1992. Prolongation of the survival of murine cardiac allografts by the vitamin D3 analogue 1,25-dihydroxy-delta 16-cholecalciferol. Transplantation 54, 762-763.

Li, Y.C., 2010. Renoprotective effects of vitamin D analogs. Kidney Int 76, 134-139.

Liu, M., Lee, M.H., Cohen, M., Bommakanti, M. and Freedman, L.P., 1996. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes Dev 10, 142-153.

Liu, P.T., Stenger, S., Li, H., Wenzel, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., Kamen, D. L. and Wagner, M., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311, 1770-1773.

Lobo, P.I., Cortez, M.S., Stevenson, W. and Pruett, T.L., 1995. Normocalcemic hyperparathyroidism associated with relatively low 1:25 vitamin D levels post-renal transplant can be successfully treated with oral calcitriol. Clin. Transplant 9, 277-281.

London, G.M., Guérin, A.P., Verbeke, F.H., Pannier, B., Boutouyrie, P., Marchais, S.J. and Mëtivier, F., 2007. Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. J Am Soc Nephrol 18, 613-620.

Lowery, E.M., Bemiss, B., Cascino, T., Durazo-Arvizu, R.A., Forsythe, S.M., Alex, C., Laghi, F., Love, R.B. and Camacho, P., 2012. Low vitamin D levels are associated with increased rejection and infections after lung transplantation. J Heart Lung Transplant 31, 700-707.

Teng, M., Wolf, M., Lowrie, E., Ofsthun, N., Lazarus, J.M. and Thadhani, R., 2003. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. N Engl J Med 349, 446-456.

Maestro, B., Dávila, N., Carranza, M.C. and Calle, C., 2003. Identification of a Vitamin D response element in the human insulin receptor gene promoter. J Steroid Biochem Mol Biol 84, 223-230.

Mantell, D.J., Owens, P.E., Bundred, N.J., Mawer, E.B. and Canfield, A.E., 2000. 1 alpha, 25-dihydroxyvitamin D(3) inhibits angiogenesis *in vitro* and *in vivo*. Circ Res 87, 214-220.

Martineau, A.R., Timms, P.M., Bothamley, G.H., Hanifa, Y., Islam, K., Claxton, A.P., Packe, G.E., Moore-Gillon, J.C., Darmalingam, M., Davidson, R.N., Milburn, H.J., Baker, L.V., Barker, R.D., Woodward, N.J., Venton, T.R., Barnes, K.E., Mullett, C.J., Coussens, A.K., Rutterford, C.M., Mein, C.A., Davies, G.R., Wilkinson, R.J., Nikolayevskyy, V., Drobniewski, F.A., Eldridge, S.M. and Griffiths, C.J., 2011. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 377, 242-250.

Mitri, J., Dawson-Hughes, B., Hu, F.B. and Pittas, A.G., 2011. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial.Am J Clin Nutr. 94, 486-494.

Montori, V.M., Basu, A., Erwin, P.J., Velosa, J.A., Gabriel, S.E. and Kudva, Y.C., 2002. Posttransplantation diabetes: a systematic review of the literature. Diabetes Care 25, 583.

Mora, J.R., Iwata, M. and Von Andrian, U.H., 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 8, 685-698.

Nagpal, J., Pande, J.N. and Bhartia, A., 2009. A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. Diabet Med 26, 19-27.

Nakagawa, K., Sasaki, Y., Kato, S., Kubodera, N. and Okano, T., 2005. 22-oxa-1alpha,25-dihydroxyvitamin D3 inhibits metastasis and angiogenesis in lung cancer. Carcinogenesis 26, 1044-1054.

Naves-Diaz, M., Alvarez-Hernandez, D., Passlick-Deetjen, J., Guinsburg, A., Marelli, C., Rodriguez-Puyol, D. and Cannata-Andia, J.B., 2008. Oral active vitamin D is associated with improved survival in hemodialysis patients. Kidney Int 74, 1070-1078.

Nibbelink, K.A., Tishkoff, D.X., Hershey, S.D., Rahman, A. and Simpson, R.U., 2007. 1,25(OH)2-vitamin D3 actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. J Steroid Biochem Mol Biol 103, 533-537.

Norman, A.W., Frankel, J.B., Heldt, A.M. and Grodsky, G.M., 1980. Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 209, 823-825.

O'Connell, T.D., Berry, J.E., Jarvis, A.K., Somerman, M.J. and Simpson, R.U., 1997. 1,25-dihydroxyvitamin D3 regulation of cardiac myocyte proliferation and hypertrophy. Am J Physiol 272, H1751-H1758.

O'Herrin, J.K., Hullett, D.A., Heisey, D.M., Sollinger, H.W. and Becker, B.N., 2002. A retrospective evaluation of 1,25-dihydroxyvitamin D(3) and its potential effects on renal allograft function. Am J Nephrol 22, 515-520.

Ohta, M., Okabe, T., Ozawa, K., Urabe, A. and Takaku, F., 1985. 1 alpha,25-dihydroxyvitamin D3 (calcitriol) stimulates proliferation of human circulating monocytes *in vitro*. FEBS Lett 185, 9-13.

Palmer, S.C., McGregor, D.O. and Strippoli, G.F., 2007. Interventions for preventing bone disease in kidney transplant recipients. Cochrane Database Syst Rev, CD005015.

Pande, S., Ritter, C.S., Rothstein, M., Wiesen, K., Vassiliadis, J., Kumar, R., Schiavi, S.C., Slatapolsky, E. and Brown, A.J., 2006. FGF-23 and sFRP-4 in chronic kidney disease and post-renal transplantation. Nephron Physiol 104, 23-32.

Park, J.W., Bae, E.H., Kim, I.J., Ma, S.K., Choi, C., Lee, J. and Kim, S.W., 2010. Paricalcitol attenuates cyclosporine-induced kidney injury in rats. Kidney Int 77, 1076-1085.

Pascussi, J.M., Robert, A., Nguyen, M., Walrant-Debray, O., Garabedian, M., Martin, P., Pineau, T., Saric, J., Navarro, F., Maurel, P. and Vilarem, M.J., 2005. Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. J Clin Invest 115, 177-186.

Penna, G., Amuchastegui, S., Giarratana, N., Daniel, K.C., Vulcano, M., Sozzani, S. and Adorini, L., 2007. 1,25-dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. J Immunol 178, 145-153.

Pilz, S., Dobnig, H., Nijpels, G., Heine, R.J., Stehouwer, C.D., Snijder, M.B., Van Dam, R.M. and Dekker, J.M., 2009. Vitamin D and mortality in older men and women. Clin Endocrinol (Oxf) 71, 666-672.

Pilz, S., Marz, W., Wellnitz, B., Seelhorst, U., Fahrleitner-Pammer, A., Dimai, H.P., Boehm, B.O. and Dobnig, H., 2008. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab 93, 3927-3935.

Pittas, A.G., Harris, S.S., Stark, P.C. and Dawson-Hughes, B., 2007a. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 30, 980-986.

Pittas, A.G., Lau, J., Hu, F.B. and Dawson-Hughes, B., 2007b. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab 92, 2017-2029.

Polesel, J., Talamini, R., Montella, M., Parpinel, M., Dal Maso, L., Crispo, A., Crovatto, M., Spina, M., La Vecchia, C. and Franceschi, S., 2006. Linoleic acid, vitamin D and other nutrient intakes in the risk of non-Hodgkin lymphoma: an Italian case-control study. Ann Oncol 17, 713-718.

Qiao, G., Kong, J., Uskokovic, M. and Li, Y.C., 2005. Analogs of 1alpha,25-dihydroxyvitamin D(3) as novel inhibitors of renin biosynthesis. J Steroid Biochem Mol Biol 96, 59-66.

Querings, K., Girndt, M., Geisel, J., Georg, T., Tilgen, W. and Reichrath, J., 2006. 25-hydroxyvitamin D deficiency in renal transplant recipients. J Clin Endocrinol Metab 91, 526-529.

Ravani, P., Malberti, F., Tripepi, G., Pecchini, P., Cutrupi, S., Pizzini, P., Mallamaci, F. and Zoccali, C., 2009. Vitamin D levels and patient outcome in chronic kidney disease. Kidney Int 75, 88-95.

Reichel, H., Koeffler, H.P., Tobler, A. and Norman, A.W., 1987. 1 alpha,25-dihydroxyvitamin D3 inhibits gamma-interferon synthesis by normal human peripheral blood lymphocytes. Proc Natl Acad Sci U S A 84, 3385-3389.

Reichrath, J., 2012. Dermatologic management, sun avoidance and vitamin D status in organ transplant recipients (OTR). J Photochem Photobiol B 101, 150-159.

Ren, C., Qiu, M.Z., Wang, D.S., Luo, H.Y., Zhang, D.S., Wang, Z.Q., Wang, F.H., Li, Y.H., Zhou, Z.W. and Xu, R.H., 2012. Prognostic effects of 25-hydroxyvitamin D levels in gastric cancer. J Transl Med 27, 10-16.

Rigby, W.F., Yirinec, B., Oldershaw, R.L. and Fanger, M.W., 1987. Comparison of the effects of 1,25-dihydroxyvitamin D3 on T lymphocyte subpopulations. Eur J Immunol 17, 563-566.

Sadlier, D.M. and Magee, C.C., 2007. Prevalence of 25(OH) vitamin D (calcidiol) deficiency at time of renal transplantation: a prospective study. Clin Transplant 21, 683-688.

Saito, H., Kusano, K., Kinosaki, M., Ito, H., Hirata, M., Segawa, H., Miyamoto, K. and Fukushima, N., 2003. Human fibroblast growth factor-23 mutants suppress Na+-dependent phosphate co-transport activity and 1alpha,25-dihydroxyvitamin D3 production. J Biol Chem 278, 2206-2211.

Sarnak, M.J., Levey, A.S., Schoolwerth, A.C., Coresh, J., Culleton, B., Hamm, L.L., McCullough, P.A., Kasiske, B.L., Kelepouris, E., Klag, M.J., Parfrey, P., Pfeffer, M., Raij, L., Spinosa, D.J., Wilson, P.W. and the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention, 2003. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the american heart association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. Hypertension 42, 1050-1065.

Schleithoff, S.S., Zittermann, A., Tenderich, G., Berthold, H.K., Stehle, P. and Koerfer, R., 2006. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 83, 754-759.

Schwarz, U., Amann, K., Orth, S.R., Simonaviciene, A., Wessels, S. and Ritz, E., 1998. Effect of 1,25 (OH)2 vitamin D3 on glomerulosclerosis in subtotally nephrectomized rats. Kidney Int 53, 1696-1705.

Scragg, R., Sowers, M. and Bell, C., 2004. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 27, 2813-2818.

Segal, E., Baruch, Y., Kramsky, R., Raz, B. and Ish-Shalom, S., 2001. Vitamin D deficiency in liver transplant patients in Israel. Transplant Proc 33, 2955-2956.

Sezer, S., Uyar, M., Arat, Z., Ozdemir, F.N. and Haberal, M., 2005. Potential effects of 1,25-dihydroxyvitamin D3 in renal transplant recipients. Transplant Proc 37, 3109-3111.

Sgambat, K., Tuchman, S., Ryan, L., Wood, R. and Moudgil, A., 2011. Low bone mineral density and nutritional vitamin D deficiency in pediatric renal transplant recipients: assessment of risk factors and response to oral vitamin D therapy. Pediatr Transplant 15, 790-797.

Shoben, A.B., Rudser, K.D., De Boer, I.H., Young, B. and Kestenbaum, B., 2008. Association of oral calcitriol with improved survival in nondialyzed CKD. J Am Soc Nephrol 19, 1613-1619.

Shroff, R., Knott, C., Gullett, A., Wells, D., Marks, S.D. and Rees, L., 2011. Vitamin D deficiency is associated with short stature and may influence blood pressure control in paediatric renal transplant recipients. Pediatr Nephrol 26, 2227-2233.

Sigmund, C.D., 2002. Regulation of renin expression and blood pressure by vitamin D(3). J Clin Invest 110, 155-156.

Soni, L.K., Hou, L., Gapstur, S.M., Evens, A.M., Weisenburger, D.D. and Chiu, B.C., 2007. Sun exposure and non-Hodgkin lymphoma: a population-based, case-control study. Eur J Cancer 43, 2388-2395.

St Peter, W.L., Li, S., Liu, J., Gilbertson, D.T., Arneson, T.J. and Collins, A.J., 2009. Effects of monthly dose and regular dosing of intravenous active vitamin D use on mortality among patients undergoing hemodialysis. Pharmacotherapy 29, 154-164.

Stavroulopoulos, A., Cassidy, M.J., Porter, C.J., Hosking, D.J. and Roe, S.D., 2007. Vitamin D status in renal transplant recipients. Am J Transplant 7, 2546-2552.

Stein, E.M. and Shane, E., 2011. Vitamin D in organ transplantation. Osteoporos Int 22, 2107-2118.

Stein, E.M., Cohen, A., Freeby, M., Rogers, H., Kokolus, S., Scott, V., Mancini, D., Restaino, S., Brown, R., McMahon, D.J. and Shane, E., 2009. Severe vitamin D deficiency among heart and liver transplant recipients. Clin Transplant 23, 861-865.

Sun, J., Kong, J., Duan, Y., Szeto, F.L., Liao, A., Madara, J.L. and Li, Y.C., 2006. Increased NF-kappaB activity in fibroblasts lacking the vitamin D receptor. Am J Physiol Endocrinol Metab 291, E315-322.

Sun, X. and Zemel, M.B., 2007. Calcium and 1,25-dihydroxyvitamin D3 regulation of adipokine expression. Obesity (Silver Spring) 15, 340-348.

Tan, X., Li, Y. and Liu, Y., 2006. Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. J Am Soc Nephrol 17, 3382-3393.

Tanaci, N., Karakose, H., Guvener, N., Tutuncu, N.B., Colak, T. and Haberal, M., 2003. Influence of 1,25-dihydroxyvitamin D3 as an immunomodulator in renal transplant recipients: a retrospective cohort study. Transplant Proc 35, 2885-2887.

Tanaka, Y., Seino, Y., Ishida, M., Yamaoka, K., Yabuuchi, H., Ishida, H., Seino, S., Seino, Y. and Imura, H., 1984. Effect of vitamin D3 on the pancreatic secretion of insulin and somatostatin. Acta Endocrinol (Copenh) 105, 528-533.

Tangpricha, V., Flanagan, J.N., Whitlatch, L.W., Tseng, C.C., Chen, T.C., Holt, P.R., Lipkin, M.S. and Holick, M.F., 2001. 25-hydroxyvitamin D-1alpha-hydroxylase in normal and malignant colon tissue. Lancet 357, 1673-1674.

Tarcin, O., Yavuz, D.G., Ozben, B., Telli, A., Ogunc, A.V., Yuksel, M., Toprak, A., Yazici, D., Sancak, S., Deyneli, O. and Akalin, S., 2009. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 94, 4023-4030.

Teng, M., Wolf, M., Lowrie, E., Ofsthun, N., Lazarus, J.M. and Thadhani, R., 2003. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. N Engl J Med 349, 446-456.

Tentori, F., Hunt, W.C., Stidley, C.A., Rohrscheib, M.R., Bedrick, E.J., Meyer, K.B., Johnson, H.K. and Zager, P.G.; Medical Directors of Dialysis Clinic Inc, 2006. Mortality risk among hemodialysis patients receiving different vitamin D analogs. Kidney Int 70, 1858-1865.

Thiem, U. and Borchhardt, K., 2011. Vitamin D in solid organ transplantation with special emphasis on kidney transplantation. Vitam Horm 86, 429-468.

Thiem, U., Heinze, G., Segel, R., Perkmann, T., Kainberger, F., Mühlbacher, F., Hörl, W. and Borchhardt, K., 2009. VITA-D: cholecalciferol substitution in vitamin D deficient kidney transplant recipients: a randomized, placebo-controlled study to evaluate the post-transplant outcome. Trials 10, 36-44.

Tishkoff, D.X., Nibbelink, K.A., Holmberg, K.H., Dandu, L. and Simpson, R.U., 2008. Functional vitamin D receptor (VDR) in the t-tubules of cardiac myocytes: VDR knockout cardiomyocyte contractility. Endocrinology 149, 558-564.

Torres, A., Garcia, S., Gomez, A., Gonzalez, A., Barrios, Y., Concepcion, M. T., Hernandez, D., Garcia, J. J., Checa, M. D., Lorenzo, V. and Salido, E., 2004. Treatment with intermittent calcitriol and calcium reduces bone loss after renal transplantation. Kidney Int 65, 705-712.

Tretli, S., Hernes, E., Berg, J.P., Hestvik, U.E. and Robsahm, T.E., 2009. Association between serum 25(OH)D and death from prostate cancer. Br J Cancer 100, 450-454.

Tuohimaa, P., Tenkanen, L., Ahonen, M., Lumme, S., Jellum, E., Hallmans, G., Stattin, P., Harvei, S., Hakulinen, T., Luostarinen, T., Dillner, J., Lehtinen, M. and Hakama, M., 2004. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: A longitudinal, nested case-control study in the Nordic countries. Int J Cancer 108, 104-108.

Tworoger, S.S., Lee, I.M., Buring, J.E., Rosner, B., Hollis, B.W. and Hankinson, S.E., 2007. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of incident ovarian cancer. Cancer Epidemiol Biomarkers Prev 16, 783-788.

Van Etten, E. and Mathieu, C., 2005. Immunoregulation by 1, 25-dihydroxyvitamin D3: Basic concepts. J Steroid Biochem Mol Biol 97, 93-101.

Veldman, C.M., Cantorna, M.T. and DeLuca, H.F., 2000. Expression of 1, 25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys 374, 334-338.

Vieth, R., Chan, P.C. and MacFarlane, G.D., 2001. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. Am J Clin Nutr 73, 288-294.

Wactawski-Wende, J., Kotchen, J.M., Anderson, G.L., Assaf, A.R., Brunner, R.L., O'Sullivan, M.J., Margolis, K.L., Ockene, J.K., Phillips, L., Pottern, L., Prentice, R.L., Robbins, J., Rohan, T.E., Sarto, G.E., Sharma, S., Stefanick, M.L., Van Horn, L., Wallace, R.B., Whitlock, E., Bassford, T., Beresford, S.A., Black, H.R., Bonds, D.E., Brzyski, R.G., Caan, B., Chlebowski, R.T., Cochrane, B., Garland, C., Gass, M., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Jackson, R.D., Johnson, K.C., Judd, H., Kooperberg, C.L., Kuller, L.H., LaCroix, A.Z., Lane, D.S., Langer, R.D., Lasser, N.L., Lewis, C.E., Limacher, M.C. and Manson, J.E.; Women's Health Initiative Investigators, 2006. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 354, 684-696.

Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M. and Vasan, R.S., 2008. Vitamin D deficiency and risk of cardiovascular disease. Circulation 117, 503-511.

Wissing, K.M., Broeders, N., Moreno-Reyes, R., Gervy, C., Stallenberg, B. and Abramowicz, D., 2005. A controlled study of vitamin D3 to prevent bone loss in renal-transplant patients receiving low doses of steroids. Transplantation 79, 108-115.

Witham, M.D., Nadir, M.A. and Struthers, A.D., 2009. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. J Hypertens 27, 1948-1954.

Wu, J., Garami, M., Cheng, T. and Gardner, D.G., 1996. 1,25(OH)2 vitamin D3, and retinoic acid antagonize endothelin-stimulated hypertrophy of neonatal rat cardiac myocytes. J Clin Invest 97, 1577-1588.

Zeitz, U., Weber, K., Soegiarto, D.W., Wolf, E., Balling, R. and Erben, R.G., 2003. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. FASEB J 17, 509-511.

Zhang, A.B., Zheng, S.S., Jia, C.K. and Wang, Y., 2003. Effect of 1,25-dihydroxyvitamin D3 on preventing allograft from acute rejection following rat orthotopic liver transplantation. World J Gastroenterol 9, 1067-1071.

Zhang, Z., Sun, L., Wang, Y., Ning, G., Minto, A.W., Kong, J., Quigg, R.J. and Li, Y.C., 2008. Renoprotective role of the vitamin D receptor in diabetic nephropathy. Kidney Int 73, 163-171.

Zhou, C., Lu, F., Cao, K., Xu, D., Goltzman, D. and Miao, D., 2008. Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. Kidney Int 74, 170-179.

Zittermann, A., 2003. Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr 89, 552-572.

Zittermann, A., Schleithoff, S.S., Götting, C., Fuchs, U., Kuhn, J., Kleesiek, K., Tenderich, G. and Koerfer, R., 2009. Calcitriol deficiency and 1-year mortality in cardiac transplant recipients. Transplantation 87, 118-124.

Zittermann, A., Schleithoff, S.S. and Koerfer, R., 2005. Putting cardiovascular disease and vitamin D insufficiency into perspective. Br J Nutr 94, 483-492.

Zittermann, A., Schleithoff, S.S. and Koerfer, R., 2007. Vitamin D and vascular calcification. Curr Opin Lipidol 18, 41-46.

Key facts

- Vitamin D insufficiency and poor bone health are common among adults and children with epilepsy.
- Multiple factors influence vitamin D levels among people with epilepsy, including nutritional status and mobility impairment, as well as choice of and duration of epilepsy treatments.
- Hepatic enzyme-inducing antiseizure medications can augment vitamin D metabolism, leading to lower vitamin D levels. However, enzyme inhibitors have also been associated with vitamin D insufficiency among people with epilepsy.
- Supplementation can improve vitamin D status, and may improve bone health among people with epilepsy.

Summary points

- Bone health is often suboptimal among people with epilepsy, and this can lead to osteoporosis and increased risk for fractures.
- Vitamin D status is one of several contributors to bone health for people with epilepsy. Overall nutrition status, antiseizure medications and dietary therapies, concurrent physical disabilities, exercise, and exposure to sunlight are also contributing factors.
- Some antiseizure medicines accelerate liver enzyme function, which leads to decreased vitamin D levels. However, even medicines that do not increase liver enzyme function have been associated with poor bone health among people with epilepsy.
- Compared to monotherapy, use of more than one antiseizure medication has been associated with higher risk for vitamin D insufficiency and lower bone mineral density.
- We suggest guidelines for screening and treatment for vitamin D insufficiency among people with epilepsy.

30. Vitamin D and bone health among people with epilepsy

R.A. Shellhaas, R.K. Singh and S.M. Joshi
University of Michigan, Department of Pediatrics & Communicable Diseases, Pediatric Neurology, C.S. Mott Children's Hospital, room 12-733, 1540 E. Hospital Drive, Ann Arbor, MI 48109-4279, USA; shellhaa@med.umich.edu

Abstract

Both adults and children with epilepsy are at high risk for poor bone health, with associated osteopenia and fractures. Epilepsy has consistently been associated with low vitamin D levels, a key indicator of bone health. Many anticonvulsant medications, such as phenytoin and carbamazepine, induce hepatic CYP450 enzymes, resulting in accelerated vitamin D metabolism. This might be the mechanism by which some epilepsy treatments decrease vitamin D levels. However, even medications which result in less-potent CYP450 induction (e.g. oxcarbazepine), or inhibit hepatic enzymes (e.g. valproate), have been associated with abnormal vitamin D levels and poor bone health. Data are lacking for many of the newer anticonvulsant medications. Although vitamin D is only one among many risk factors for abnormal bone mineral density, it is a modifiable risk factor for people with epilepsy. We suggest guidelines for screening and treatment of vitamin D insufficiency for people with epilepsy.

Keywords: epilepsy, seizure, bone health, anticonvulsant, antiepileptic drug, pediatric

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_30, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
BMD	Bone mineral density
CI	Confidence interval
OR	Odds ratio

30.1 Introduction

Epilepsy is one of the most common neurological disorders, affecting about 1% of all people (Faught *et al.*, 2012; Russ *et al.*, 2012). Although unprovoked seizures are the hallmark of epilepsy, there are numerous physical and mental health comorbidities. Herein we address the complex associations between vitamin D, bone health, and epilepsy, including contributions related to patients' environmental exposures, medical treatments, and diet.

30.2 Epilepsy terminology

Epilepsy is, by definition, a condition of recurrent, unprovoked seizures. There are many epilepsy types and numerous distinct epilepsy syndromes. The details of epilepsy classification are beyond the scope of this chapter, but a basic understanding of epilepsy terminology will allow the reader to better understand the literature reviewed below.

Seizures are loosely classified as being *focal* or *generalized*. Focal seizures (also called partial seizures) originate in one area of the brain and may spread to contiguous or contralateral regions. Focal seizures may or may not result in impaired awareness. Generalized seizures originate within bilaterally distributed neural networks and so involve both cerebral hemispheres. Types of generalized seizures include absence, generalized tonic-clonic (convulsions), atonic (drop seizures), myoclonic, etc. Usually, patients experience either focal or generalized seizures, although a small subset of patients has both types. In the literature, epilepsy is often referred to as 'well-controlled', meaning the patient is seizure-free, or 'treatment-resistant'. Patients are considered to have treatment-resistant epilepsy if their seizures that are not controlled despite adequate trials of two appropriately-chosen and well-tolerated antiseizure drugs (Kwan *et al.*, 2010).

Epilepsy syndromes can be focal (also called partial or localization-related epilepsies) or generalized, depending on the associated seizure type(s). Historically, epilepsy has been further classified into *symptomatic* and *idiopathic* categories. Symptomatic epilepsies have an identifiable trigger (e.g. a patient who developed recurrent focal seizures after sustaining a stroke would be classified as having symptomatic focal epilepsy). The term *idiopathic* does not mean 'unknown' in epilepsy parlance. Rather, *idiopathic* refers to epilepsy syndromes that are presumed to have a genetic etiology. Typically, the idiopathic epilepsy syndromes are thought to be more benign

than the symptomatic epilepsy syndromes. Examples of idiopathic epilepsies include childhood absence epilepsy and benign Rolandic epilepsy. Note that newer schemes for organizing the epilepsy syndromes are being proposed and are slowly being adopted (Berg *et al.*, 2010), but the literature regarding vitamin D and epilepsy uses the above-defined terminology.

30.3 Prevalence of vitamin D insufficiency among people with epilepsy

The association between vitamin D insufficiency, treatment with antiseizure drugs, and poor bone health among people with epilepsy has been recognized for decades and the American Academy of Pediatrics has highlighted children taking antiseizure medications as a high-risk group (Wagner *et al.*, 2008). The reported prevalence of vitamin D insufficiency among people with epilepsy is variable, depending on the definition of vitamin D insufficiency, the time of the year during which the data were collected, the geographical location of the study population, and the population actually studied. However, all published data report that a significant proportion of patients with epilepsy have insufficient 25(OH)D levels and this trend does not appear to have improved over the years. Both children and adults with well-controlled and treatment-resistant epilepsy are at risk.

In the 1970's, studies of institutionalized children with epilepsy and mental retardation showed abnormal serum markers for rickets, which correlated with duration of antiseizure drug therapy (Lifshitz and Maclaren, 1973). Children with epilepsy (age 10-16 years) were also found to have much higher rates of insufficient vitamin D (defined then as <15 ng/ml) than healthy controls (72% vs. 50%), with seasonal variability such that levels were highest during the summer months and lowest in the winter (Offermann *et al.*, 1979).

Modern data suggest similar rates of vitamin D insufficiency among children with epilepsy. In a German study, 76% of a cohort of 33 children with a variety of epilepsy syndromes had 25(OH)D <20 ng/ml, compared to 23% of 44 healthy controls (P<0.001) (Nettekoven *et al.*, 2008). Shellhaas and coworkers observed that in an unselected group of American children with epilepsy, 25% of 78 subjects had a 25(OH)D level <20 ng/ml (Shellhaas *et al.*, 2010). More significantly, only 25% of the children had levels that were considered to be normal (25(OH)D >32 ng/ml).

Insufficient vitamin D levels have also been reported for adults with epilepsy. In a Brazilian study, 34% of individuals with epilepsy (without comorbid mental retardation), who were treated with antiseizure medications for 2-38 years, had insufficient 25(OH)D levels, compared with 7% of controls (P<0.02) (Kulak *et al.*, 2004). Lower levels of 25(OH)D were reported by Pedera and coworkers among 30 adults with epilepsy who were seizure-free on antiseizure medications, compared to 30 controls (22.0±12.1 ng/ml vs. 31.2±8.6 ng/ml, P<0.0001). The low 25(OH)D levels could be corrected with vitamin D supplementation (Pedrera *et al.*, 2000).

30.4 Epilepsy and fracture risk

The reasons adults and children with epilepsy are at significant risk for fractures are multifactorial. Vitamin D status certainly plays a role, but calcium intake, choice of antiseizure medication(s), use of polypharmacy, duration of treatment, comorbid abnormal ambulatory status, as well as the seizures themselves, all contribute to this risk.

Women with epilepsy appear to be at particularly increased risk for bone fractures (Espinosa *et al.*, 2011; Souverein *et al.*, 2006), which might not be ameliorated by standard calcium and vitamin D supplementation. A large cohort of Caucasian women 65 or older was evaluated prospectively for about 4 years to determine the incidence of hip fracture (Cummings *et al.*, 1995). In this group, concurrent use of antiseizure drugs was associated with a relative risk for hip fracture of 2.8 (95% CI 1.2-6.3) compared with those not presently taking antiseizure medication. None of the hip fractures that occurred in the women taking antiseizure medications were directly attributable due to seizures or loss of consciousness.

Adults with epilepsy are at higher risk for fractures than those with many other chronic illnesses (OR 2.89 compared with healthy controls), with the highest risk among those treated with multiple antiseizure drugs. Although valproic acid might have a lower associated fracture risk, all other antiseizure medications have been associated with increased risk for non-traumatic fractures among adults older than 50 years of age (Jetté *et al.*, 2011). The same conclusions hold true for children with epilepsy. The risk of fractures among children who take anticonvulsants is 2-6 times greater than the general population (Mattson and Gidal, 2004).

30.5 Risk factors for poor bone health among people with epilepsy

Numerous aspects of epilepsy are likely to influence vitamin D levels and bone health among affected individuals, as summarized in Table 30.1 and detailed below.

30.5.1 Nutritional status

Children with treatment-resistant epilepsy are at risk of poor nutritional status, lacking adequate protein, vitamin, and mineral intake (Volpe *et al.*, 2007). However, inadequate dietary calcium and vitamin D is not restricted to those with the most severe epilepsy syndromes. In a longitudinal study in India, 144 patients with new onset epilepsy who were not yet treated with antiseizure medications were observed over a 6 month period (Menon and Harinarayan, 2010). All patients in this study had lower than recommended dietary calcium intake. Over 90% of this cohort had low 25(OH)D levels at the time of their epilepsy diagnosis and showed a significant decline in 25(OH)D levels over 6 months of treatment, regardless of the medication prescribed. Thus the need for antiseizure medications is associated with further compromise of vitamin D status.

Table 30.1. Epilepsy-related factors associated with vitamin D insufficiency and poor bone health.

Nutritional status	Impaired mobility	Environmental light exposure	Epilepsy treatments[1]
Inadequate dietary vitamin D and calcium intake Obesity	Co-morbid cerebral palsy and other neuro-motor abnormalities	Geographical location Limited outdoor physical exercise Home versus institutional living arrangements	Antiseizure medications • CYP450 enzyme induction • Monotherapy vs. polypharmacy Ketogenic diet

[1] Vitamin D receptor polymorphisms may compound the risk (Lambrinoudaki *et al.*, 2011).

Obesity is a major identified risk factor for vitamin D insufficiency in the general population, both for adults and children (Looker *et al.*, 2008; Reid *et al.*, 2009; Saintonge *et al.*, 2009). Recently, obesity has been identified as a common co-morbidity among children with newly diagnosed epilepsy, particularly among adolescents and those with idiopathic epilepsy syndromes (Daniels *et al.*, 2009). Higher body mass index was reported as a risk factor for lower vitamin D levels in a cohort of 78 children with epilepsy (Shellhaas *et al.*, 2010). Since people with obesity will require higher vitamin doses to reach an appropriate 25(OH)D level, particular attention should be paid to this risk factor.

30.5.2 Impaired mobility

A subset of individuals with epilepsy has impaired mobility due to co-morbid neurological conditions, such as cerebral palsy, which can affect ambulatory status and impact bone health. Most published studies have specifically excluded non-ambulatory patients with epilepsy, so there are limited data to assess the contribution of ambulation status on bone health among those with epilepsy. In a large study of children (with and without epilepsy), the risk of vitamin D deficiency among nonambulatory children was about twice that of ambulatory children, even after adjusting for confounders. That study also found depressed BMD *z*-scores among nonambulatory patients, independent of antiseizure drug status (Baer *et al.*, 1997).

Others reported no effects of ambulatory status on 25(OH)D levels among noninstitutionalized children with treatment-resistant epilepsy, although they may have been underpowered to detect such a difference (Bergqvist *et al.*, 2007). Even among children with normal ambulatory status, those with epilepsy exercise less than their sibling controls (Wong and Wirrell, 2006), suggesting that modifiable risk factors for poor bone health are readily identifiable for many patients.

30.5.3 Physical environment and light exposure

Only 10% of vitamin D is obtained through dietary sources. The rest, and therefore vast majority of vitamin D, is generated via cutaneous synthesis, following exposure to sunlight. Individuals with epilepsy are known to have lower levels of physical activity than healthy controls. Physical activity and outdoor sun exposure may be further limited among children with epilepsy and comorbid conditions, such as cerebral palsy, since these children are often wheelchair bound and not ambulatory. Wong and coworkers demonstrated low vitamin D levels in virtually all of 122 institutionalized patients, regardless of their antiseizure drug regimen (Wong *et al.*, 2006). It was notable that sun exposure in this cohort was minimal, or nonexistent.

There was a notable difference in sunlight exposure among 38 German children with a variety of epilepsy syndromes, compared with 44 healthy controls (66% vs. 100% receiving daily sunlight exposure, $P<0.001$) (Nettekoven *et al.*, 2008). Sun exposure varies based on the time of the year and season. Similarly vitamin D levels fluctuate during the year, both for healthy people (Looker *et al.*, 2008) and those with epilepsy (Nettekoven *et al.*, 2008; Offermann *et al.*, 1979). Additional vitamin D supplementation should be considered during seasons with lower sunlight.

30.6 Epilepsy treatments

Because many antiseizure drugs induce hepatic CYP450 metabolism, they can result in accelerated metabolism of vitamin D, leading to declining 25(OH)D levels, increased parathyroid hormone levels, and abnormally enhanced bone turnover. However, hepatic enzyme induction is only one component of the complex mechanisms by which antiepileptic drugs affect bone health. There was no significant difference in BMD reduction among men and women treated with enzyme-inducing medications compared with those receiving non-inducing antiseizure drugs in one recent study (Farhat *et al.*, 2002). Additionally, 25(OH)D levels were reduced among patients treated with the powerful enzyme-inducer carbamazepine, as well as among those taking the less-potent inducer oxcarbazepine (Mintzer *et al.*, 2006).

Studies have consistently demonstrated that treatment with a single antiseizure medication is less deleterious for vitamin D status and bone health than polytherapy (Bergqvist *et al.*, 2007; El-Hajj Fuleihan *et al.*, 2008; Jetté *et al.*, 2011; Nettekoven *et al.*, 2008). However, patients who require polytherapy generally have treatment-resistant epilepsy syndromes and so are more likely to have comorbid impaired mobility, nutritional deficiencies, and suboptimal sunlight exposure. Below, we examine the impact of specific epilepsy treatments on vitamin D and bone health.

30.6.1 Phenytoin, primidone, and phenobarbital

Adults

The antiseizure medications most commonly associated with altered bone metabolism and decreased bone density are inducers of the cytochrome P450 enzyme system, and include phenytoin, primidone, and phenobarbital (Gough *et al.*, 1986; O'Hare *et al.*, 1980; Pack *et al.*, 2008; Valimaki *et al.*, 1994). Phenytoin is associated with increased bone turnover, as demonstrated by elevated markers of bone resorption and bone formation among adults (Pack *et al.*, 2008; Valimaki *et al.*, 1994). After one year of treatment, women taking phenytoin were found to have significant BMD loss at the femoral neck, as well as decreased 25(OH)D and other markers of abnormal bone turnover (Pack *et al.*, 2008).

Children

The effects of these older antiepileptic medications on bone health have not been evaluated extensively in the pediatric population, since in contemporary practice phenytoin and primidone are not often prescribed for children. Phenobarbital is commonly prescribed for infants with epilepsy, but its effects on these patients' vitamin D status have not been studied.

30.6.2 Carbamazepine

Adults

Like phenobarbital and phenytoin, carbamazepine is potent inducer of the cytochrome P450 enzyme system. However, studies evaluating its effect on vitamin D and BMD in adults have been conflicting, with some reporting significant disturbances in bone and mineral metabolism and bone turnover, and others finding no such abnormalities. Normal 25(OH)D levels were reported among 53 young women treated with carbamazepine (Pack *et al.*, 2011). In contrast, people taking carbamazepine (n=21) were found to have lower 25(OH)D levels than normal controls (n=42) (Mintzer *et al.*, 2006).

Among adults treated with carbamazepine monotherapy, BMD was not significantly decreased (Valimaki *et al.*, 1994). However, patients treated with carbamazepine monotherapy have demonstrated decreased cortical bone mass, as measured by quantitative ultrasonography of the phalanges (Pedrera *et al.*, 2000; Pluskiewicz and Nowakowska, 1997). The differing results suggest that the effects of carbamazepine monotherapy on bone and mineral metabolism and BMD have not yet been completely delineated.

Children

Several groups have examined vitamin D levels among children treated with carbamazepine. Two teams reported no statistically significant differences between 25(OH)D levels in control subjects

and carbamazepine-treated children, thus refuting the theory of hepatic enzyme induction as the driving force for hypovitaminosis D among pediatric epilepsy patients (Babayigit *et al.*, 2006; Verrotti *et al.*, 2002). Conversely, one group (Nicolaidou *et al.*, 2006) observed that 37% of ambulatory patients with idiopathic epilepsies treated with either carbamazepine or valproic acid exhibited 25(OH)D levels of <10 ng/ml in the first year of treatment, and that the mean 25(OH)D level declined over the 36-month treatment period. This study also reported a significant negative correlation between 25(OH)D and parathyroid hormone, as well as seasonal variation among 25(OH)D levels, but did not examine BMD. Similarly, a fourth group reported that although increasing carbamazepine levels were not significantly associated with changes in 25(OH)D or parathormone, 25(OH)D declined by 22%, regardless of carbamazepine dosing among those newly treated for epilepsy (Misra, 2010).

Among pediatric studies that examined BMD in relation to treatment with carbamazepine, five reported no significant difference (Akin *et al.*, 1998; Altay *et al.*, 2000; Kafali *et al.*, 1999; Sheth *et al.*, 1995; Tekgul *et al.*, 2006), whereas one reported lower BMD but unchanged 25(OH)D levels (Babayigit *et al.*, 2006) compared with healthy control subjects. Another study reported lower BMD among children receiving carbamazepine compared with those receiving valproic acid (Chou *et al.*, 2007).

30.6.3 Valproate

Adults

Since valproate is an effective treatment for many different epilepsy syndromes, thorough study of its effect on vitamin D and bone health is warranted. Valproate is an inhibitor of the cytochrome P450 enzyme system, but emerging data suggest it may still adversely affect bone health. Early reports evaluating indexes of bone metabolism among patients taking valproate found no significant abnormalities. Adults treated with valproate monotherapy had no significant reductions in calcium or 25(OH)D levels and alkaline phosphatase was not increased (Davie *et al.*, 1983; Gough *et al.*, 1986).

However, more recent studies report different results. A study of adults receiving long-term valproate monotherapy found increased serum concentrations of calcium, low levels of vitamin D metabolites, increased markers of bone resorption and formation, and decreased BMD (Sato *et al.*, 2001). The increased calcium levels were postulated to reflect increased bone resorption. Despite this, valproate was the only antiseizure drug *not* associated with increased fracture risk for older adults with epilepsy (Jetté *et al.*, 2011).

Children

Among children, the effects of valproic acid on BMD and markers of bone health are also complex. One study specifically reported vitamin D levels among children with idiopathic epilepsies compared with healthy controls and found no significant difference (Babayigit *et al.*,

2006), while another demonstrated decreasing 25(OH)D levels among children with idiopathic epilepsies (Nicolaidou *et al.*, 2006). Higher BMD has been reported among those receiving valproic acid compared with those on carbamazepine (Chou *et al.*, 2007). One study reported lower BMD among subjects treated with valproic acid and/or lamotrigine compared with control subjects (Guo *et al.*, 2001), but five studies reported no significant difference in bone mineral density among children treated with valproic acid (Akin *et al.*, 1998; Altay *et al.*, 2000; Kafali *et al.*, 1999; Sheth *et al.*, 1995; Tekgul *et al.*, 2006).

30.7 Newer antiseizure medications

Many new antiseizure drugs have been approved in the past 15 years, but few studies have evaluated the effect of these medications on 25(OH)D and BMD. Those studied include oxcarbazepine, lamotrigine, topiramate, and the ketogenic diet. Since new antiseizure medications are very commonly prescribed, due to favorable side effect profiles and superior efficacy, additional research is urgently required to determine whether any of the newer medications cause abnormalities in bone health and vitamin D metabolism.

30.7.1 Oxcarbazepine

Adults

Because recent guidelines suggest that oxcarbazepine is the initial treatment of choice for people with focal epilepsy syndromes (French *et al.*, 2004), studying this medication's effects on bone health is essential. At high doses, oxcarbazepine is a hepatic enzyme inducer, albeit less potent than carbamazepine. In the only study of adults designed specifically to evaluate oxcarbazepine, patients with focal epilepsies taking carbamazepine or oxcarbazepine were compared with healthy control subjects, and those on carbamazepine were crossed over to oxcarbazepine monotherapy (Mintzer *et al.*, 2006). 25(OH)D levels were significantly lower among the oxcarbazepine and carbamazepine subjects, compared with control subjects, but were not significantly different between the oxcarbazepine and carbamazepine groups, suggesting that the newer agent may also exert an important impact on bone health.

Children

Recently two studies evaluated the effects of oxcarbazepine on bone health in children with epilepsy. One group (Cansu *et al.*, 2008) prospectively studied 34 newly diagnosed children with idiopathic focal epilepsy who were ambulatory and without other neurologic or medical diagnoses. Patients were treated with oxcarbazepine for 18 months. Mean 25(OH)D levels were <30 ng/ml at baseline and declined significantly over the 18 months study period. Another group compared patients with idiopathic epilepsy syndromes treated with oxcarbazepine (n=14), carbamazepine (n=23), or valproic acid (n=31) to 30 healthy control children. Although vitamin

D levels were not lower among treated patients, total BMD was lower for those taking any of the anticonvulsant drugs, compared to controls (Babayigit *et al.*, 2006).

30.7.2 Lamotrigine

Adults

Data regarding the impact of lamotrigine on bone health are mixed. One prospective study found elevated osteocalcin, a marker of bone formation, in association with lamotrigine treatment (Kim *et al.*, 2007). In contrast, premenopausal women treated with lamotrigine did not have significant reductions in BMD or changes in bone turnover markers (Pack *et al.*, 2005). Recent reports found no significant reductions in 25(OH)D, calcium, or markers of bone resorption and bone formation among young women treated with lamotrigine monotherapy (Pack *et al.*, 2011).

Children

Only one published study reported the effects of valproate and lamotrigine on growth and bone mass in children with epilepsy, both alone and in combination. Fifty-three patients taking one or both of these antiseizure drugs, who were without bone or metabolic disease, other bone-altering medications, or a family history of osteoporosis, were studied. Twenty three subjects (43%) were below the 10^{th} percentile for height, and nine (24%) had a BMD *z*-score <-1.5, without significant differences between those treated with either medication. Decreased physical activity scores were associated with worse bone-health markers, including lower 25(OH)D levels. The combination of dual therapy with valproic acid and lamotrigine, in addition to low activity scores, was associated with the highest risk of low BMD and short stature(Guo *et al.*, 2001). 25(OH)D levels were normal among 14 European children with idiopathic epilepsies who were taking lamotrigine (Borusiak *et al.*, 2012).

30.7.3 Topiramate

Adults

There has been limited study of topiramate in relation to bone health. As a carbonic anhydrase inhibitor, topiramate can induce renal acidosis, with theoretical risk for secondary abnormalities of bone metabolism. One study demonstrated that although serum bicarbonate levels were lower among pre-menopausal Korean women taking topiramate than controls or those taking carbamazepine or valproate, 25(OH)D levels did not differ between these groups (Heo *et al.*, 2011). Young women taking topiramate for migraine prophylaxis had normal 1,25-dihydroxyvitamin D levels, but low BMD (25(OH)D levels were not reported) (Ali *et al.*, 2011).

Children

There are very few data regarding the effect of topiramate on bone health among children. Topiramate was associated with lower BMD among a small group of children with treatment-resistant epilepsy, taking more than one anticonvulsant (Coppola *et al.*, 2009). This medication is very commonly prescribed for a wide variety of childhood epilepsies, so additional evaluation of associated 25(OH)D and BMD changes is imperative.

30.7.4 Ketogenic diet

The ketogenic diet is a high fat, low calorie and low carbohydrate and protein diet used to treat drug-resistant epilepsy. This diet is very restrictive, and usually requires supplementation with vitamins and other micronutrients. Children on the ketogenic diet are at risk for poor bone health, and low vitamin D levels. Vitamin D levels must be evaluated serially, as 25(OH)D levels have been shown to decline by ~0.5ng/ml per month during 15-months of treatment with ketogenic diet (Bergqvist *et al.*, 2007).

Changes in BMD are also common among children treated with the ketogenic diet. On average, these children's height and weight status are low at baseline, and decline while they are on the ketogenic diet. Those at highest risk for poor BMD are younger patients who are non-ambulatory and have a low baseline body mass index (Bergqvist *et al.*, 2008).

30.8 Suggested screening and treatment for insufficient vitamin D status among people with epilepsy

Robust data to support specific vitamin D supplement regimens for people with epilepsy are lacking. However, a few intervention studies are available. Adding vitamin D to the treatment regimen of institutionalized children with cerebral palsy increased BMD, in comparison with controls not treated with vitamin D, who continued to show loss of bone mass (Jekovec-Vrhovsek *et al.*, 2000). However, a study of adults with epilepsy demonstrated that those taking calcium and vitamin D supplements were just as likely to sustain a bone fracture as those who took no supplements (Espinosa *et al.*, 2011). The only controlled trial of vitamin D supplementation (400 units daily vs. 2,000 units daily) among children with epilepsy failed to show an improvement in BMD after one year and 25(OH)D levels rose only marginally (Mikati *et al.*, 2006).

Exciting preliminary data suggest that vitamin D supplementation may have an anticonvulsant effect. In a study of 13 adult patients with treatment-resistant epilepsy (10 focal, 3 generalized), 12 of whom had comorbid vitamin D deficiency, there was a median 40% reduction in seizures after 3 months of aggressive vitamin D supplementation (Hollo *et al.*, 2012). If these data are replicated in larger patient populations, physicians will be compelled to optimize vitamin D levels for people with epilepsy.

Currently, however, we lack rigorous evidence to support screening for, or treating, vitamin D insufficiency for people with epilepsy. Despite this, a substantial body of literature suggests that vitamin D insufficiency may be important for these patients. Diagnostic testing is readily available and treatment with vitamin D supplements is relatively inexpensive and free of substantial risk.

Future research may compel us to revise our practice, but based on the available data we offer suggestions for screening and treatment of insufficient vitamin D levels for those with epilepsy (Table 30.2). We measure 25(OH)D levels for all epilepsy patients on an annual basis, if the level is >30 ng/ml. We do not routinely measure 1,25-dihydrovitamin D levels, since these are virtually always normal (Bergqvist *et al.*, 2007; Shellhaas *et al.*, 2010). For those with normal 25(OH)D levels (>30 ng/ml), we recommend a daily multivitamin containing a minimum of 400 units of vitamin D. For those whose levels are <30 ng/ml, we prescribe vitamin D supplements (Table 30.2). We recommend administering cholecalciferol (D3) rather than ergocalciferol (D2), since the former has better intestinal absorption than the latter among people with normal renal function.

Obesity is a risk factor for vitamin D insufficiency. Since vitamin D is fat soluble, higher than usual doses of supplemental cholecalciferol are needed to maintain adequate circulating 25(OH) D levels. Special consideration should also be given to adjusting supplement doses during winter or for individuals whose personal circumstances do not allow for adequate sunlight exposure.

Some epilepsy centers routinely evaluate patients with treatment-resistant epilepsy with bone density scans. There is a paucity of data to support this practice, and BMD measurement is

Table 30.2. Suggested screening and treatment for vitamin D insufficiency for people with epilepsy.

25(OH)D level (ng/ml)	Cholecalciferol dose	Repeat labs	Additional labs
≥30	400 IU per day[1]	Annually	
15 to 29	2,000 IU per day in divided doses	Every 4 weeks until 25(OH)D >30[2]	Phosphorus, magnesium, calcium
<15	2,000-4,000 IU per day in divided doses, in consultation with a dietician	Every 3-4 weeks until 25(OH)D >30[2]	Phosphorus, calcium, magnesium Parathyroid hormone Consider bone density scan

[1] In accordance with American Academy of Pediatrics recommendations, we suggest that all children treated for epilepsy be given a daily multivitamin with 400 international units of vitamin D3. Some authors have suggested a higher standard dose of 1000 international units per day (Wirrell, 2010).

[2] Once 25(OH)D levels rise to the normal range, we halve the vitamin D supplement dose. If the level remains normal, recheck in 3-6 months.

relatively expensive, so we recommend pursuing bone density measurements on a case-by-case basis, with this testing reserved for those at highest risk for osteopenia (e.g. those with history of atraumatic bone fracture and vitamin D deficiency).

30.9 Conclusions

Although the risk for poor bone health among people with epilepsy, and its potential relationships to anticonvulsants and vitamin D, has been recognized for decades, the literature remains contradictory. Many medications (especially carbamazepine, phenytoin, and phenobarbital) can increase vitamin D metabolism via induction of the CYP450 system. However, non-enzyme-inducing medications, such as valproate, and less powerful enzyme-inducers (oxcarbazepine) may also exert adverse effects on bone mineralization. Evidently, hepatic enzyme induction and vitamin D levels are not the only factors contributing to bone mineralization among people with epilepsy.

People with treatment-resistant epilepsy, typically treated with multiple antiseizure medications, are at high risk for poor bone health. This is clearly a multifactorial problem, influenced by co-morbid neuromotor disabilities, body mass index, medications, diet and vitamin D, as well as the seizures themselves. Some of these factors are modifiable, such as vitamin D intake and other dietary considerations, while others are not. Despite some contradictory evidence, we therefore advocate for attention to vitamin D levels as part of the comprehensive management for such vulnerable patients. The cost is relatively low, but the potential ramifications are tremendous.

Despite all of the attention in the lay press and scientific literature, physicians do not consistently screen for vitamin D insufficiency. More than ten years ago, a survey revealed that only 41% of pediatric neurologists and 28% of adult neurologists (all of whom were members of the American Academy of Neurology) screened for bone disease among their patients with epilepsy, with just 3% routinely monitoring parathyroid hormone or 25(OH)D levels (Valmadrid *et al.*, 2001). Ten years later, a study of English pediatric neurologists revealed that only a minority routinely considered bone health screening or prophylaxis for their patients with epilepsy (Fong *et al.*, 2011). If additional research demonstrates a beneficial effect of interventions such as vitamin D and/or calcium supplementation on bone health for people with epilepsy, we hope that these numbers, and the interest of neurologists and primary care providers in this important aspect of comprehensive epilepsy care, will rise. Until evidence-based treatment guidelines become available, we suggest consideration of screening for vitamin D insufficiency and supplementation with cholecalciferol to maintain levels in the optimal range.

References

Akin, R., Okutan, V., Sarici, U., Altunbas, A. and Gokcay, E., 1998. Evaluation of bone mineral density in children receiving antiepileptic drugs. Pediatric Neurology 19, 129-131.

Ali, I.I., Herial, N.A., Orris, M., Horrigan, T. and Tietjen, G.E., 2011. Migraine prophylaxis with topiramate and bone health in women. Headache 51, 613-616.

Altay, E.E., Serdaroglu, A., Tumer, L., Gucuyener, K. and Hasanoglu, A., 2000. Evaluation of bone mineral metabolism in children recieving carbamazepine and valproic acid. Journal of Pediatric Endocrinology & Metabolism 13, 933-939.

Babayigitm, A., Dirik, E., Bober, E. and Cakmakci, H., 2006. Adverse effects of antiepileptic drugs on bone mineral density. Pediatric Neurology 35, 177-181.

Baer, M.T., Kozlowski, B.W., Blyler, E.M., Trahms, C.M., Taylor, M.L. and Hogan, M.P., 1997. Vitamin D, calcium, and bone status in children with developmental delay in relation to anticonvulsant use and ambulatory status. American Journal of Clinical Nutrition 65, 1042-1051.

Berg, A.T., Berkovic, S.F., Brodie, M.J., Buchhalter, J.R., Cross, J.H., van Emde Boas, W., Engel, J., French, J., Glauser, T.A., Mathern, G.W., Moshe, S.L., Nordli, D., Plouin, P. and Scheffer, I.E., 2010. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 51, 676-685.

Bergqvist, A.G.C., Schall, J.I. and Stallings, V.A., 2007. Vitamin D status in children with intractable epilepsy, and impact of the ketogenic diet. Epilepsia 48, 66-71.

Bergqvist, A.G.C., Schall, J.I., Stallings, V.A. and Zemel, B.S., 2008. Progressive bone mineral content loss in children with intractable epilepsy treated with the ketogenic diet. American Journal of Clinical Nutrition 88, 1678-1684.

Borusiak, P., Langer, T., Heruth, M., Karenfort, M., Bettendorf, U. and Jenke, A.C., 2012. Antiepileptic drugs and bone metabolism in children: data from 128 patients. Journal of Child Neurology 28, 176-183.

Cansu, A., Ediz, Y., Ayse, S., Hirfanoglu, T.L., Camurdan, O., Gulbahar, O., Gucuyener, K. and Cinaz, P., 2008. Evaluation of bone turnover in epileptic children using oxcarbazepine. Pediatric Neurology 39, 266-271.

Chou, I.-J., Lin, K.-L., Wang, H.-S. and Wang, C.-J., 2007. Evaluation of bone mineral density in children receiving carbamazepine or valproate monotherapy. Acta Paediatrica Taiwanica 48, 317-322.

Coppola, G., Fortunato, D., Auricchio, G., Mainolfi, C., Operto, F.F., Signoriello, G., Pascotto, A. and Salvator, M., 2009. Bone mineral density in children, adolescents, and young adults with epilepsy. Epilepsia 50, 2140-2146.

Cummings, S.R., Nevitt, M.C., Browner, W.S., Stone, K., Ensrud, K.E., Cauley, J., Black, D. and Vogt, T.M., 1995. Risk factors for hip fracture in white women. Study of osteoporotic fractures research group. New England Journal of Medicine 332, 767-773.

Daniels, Z.S., Nick, T.G., Liu, C., Cassedy, A. and Glauser, T.A., 2009. Obesity is a common comorbidity for pediatric patients with untreated, newly diagnosed epilepsy. Neurology 73, 658-664.

Davie, M.W., Emberson, C.E., Lawson, D.E., Roberts, G.E., Barnes, J.L., Barnes, N.D. and Heeley, A.F., 1983. Low plasma 25-hydroxyvitamin D and serum calcium levels in institutionalized epileptic subjects: associated risk factors, consequences and response to treatment with vitamin D. Quarterly Journal of Medicine 52, 79-91.

El-Hajj Fuleihan, G., Dib, L., Yamout, B., Sawaya, R. and Mikati, M.A., 2008. Predictors of bone density in ambulatory patients on antiepileptic drugs. Bone 43, 149-155.

Espinosa, P.S., Perez, D.L., Abner, E. and Ryan, M., 2011. Association of antiepileptic drugs, vitamin D, and calcium supplementation with bone fracture occurrence in epilepsy patients. Clinical Neurology and Neurosurgery 113, 548-551.

Farhat, G., Yamout, B., Mikati, M.A., Demirjian, S., Sawaya, R. and El-Hajj Fuleihan, G., 2002. Effect of antiepileptic drugs on bone density in ambulatory patients. Neurology 58, 1348-1353.

Fong, C.Y., Mallick, A.A., Burren, C.P. and Patel, J.S., 2011. Evaluation and management of bone health in children with epilepsy on long-term antiepileptic drugs: United Kingdom survey of paediatric neurologists. European Journal of Pediatric Neurology 15, 417-423.

Fraught, E., Richman, J., Martin, R., Funkhouser, E., Foushee, R., Kratt, P., Kim, Y., Clements, K., Cohen, N., Adoboe, D., Knowlton, R. and Pisu, M., 2012. Incidence and prevalence of epilepsy among older US Medicare beneficiaries. Neurology 78, 448-453.

French, J.A., Kanner, A.M., Bautista, J., Abou-Khalil, B., Browne, T., Harden, C.L., Theodore, W.H., Bazil, C., Stern, J., Schachter, S.C., Bergen, D., Hirtz, D., Montouris, G.D., Nespeca, M., Gidal, B., Marks, W.J., Turk, W.R., Fischer, J.H., Bourgeois, B., Wilner, A., Faught, R.E., Sachdeo, R.C., Beydoun, A. and Glauser, T.A., 2004. Efficacy and tolerability of the new antiepileptic drugs I: Treatment of new onset epilepsy. Neurology 62, 1252-1260.

Gough, H., Goggin, T., Bissessar, A., Baker, M., Crowley, M. and Callaghan, N., 1986. A comparative study of the relative influence of different anticonvulsant drugs, UV exposure and diet on vitamin D and calcium metabolism in out-patients with epilepsy. Quarterly Journal of Medicine 230, 569-577.

Guo, C.-Y., Ronene, G.M. and Atkinson, S.A., 2001. Long-term valproate and lamotrigine treatment may be a marker for reduced growth and bone mass in children with epilepsy. Epilepsia 42, 1141-1147.

Heo, K., Rhee, Y., Lee, H.W., Lee, S.A., Shin, D.J., Kim, W.-J., Song, H.-K., Song, S. and Lee, B.I., 2011. The effect of topiramate monotherapy on bone mineral density and markers of bone and mineral metabolism in premenopausal women with epilepsy. Epilepsia 52, 1884-1889.

Hollo, A., Clemens, Z., Kamondi, A., Lakatos, P. and Szucs, A., 2012. Correction of vitamin D deficiency improves seizure control in epilepsy: a pilot study. Epilepsy & Behavior 24, 131-133.

Jekovec-Vrhovsek M, Kocijancic A and Prezelj J, 2000. Effect of vitamin D and calcium on bone mineral density in children with CP and epilepsy in full-time care. Developmental Medicine and Child Neurology 42, 403-405.

Jetté, N., Lix, L.M., Metge, C.J., Prior, H.J., McChesney, J. and Leslie, W.D., 2011. Association of antiepileptic drugs with nontraumatic fractures. Archives of Neurology 68, 107-112.

Kafali G, Erselcan T and Tanzer F, 1999. Effect of antiepileptic drugs on bone mineral density in children between ages 6 and 12 years. Clinical Pediatrics 38, 93-98.

Kim, S.H., Lee, J.W., Choi, K.G., Chung, H.W. and Lee, H.W., 2007. A 6-month longitudinal study of bone mineral density with antiepileptic drug monotherapy. Epilepsy & Behavior 10, 291-295.

Kulak, C.A., Borba, V.Z., Bilezikian, J.P., Silvado, C.E., Paola, L. and Boguszewski, C.L., 2004. Bone mineral density and serum levels of 25 OH vitamin D in chronic users of antiepileptic drugs. Arquivos de Neuro-Psiquiatria 62, 940-948.

Kwan, P., Arzimanoglou, A., Berg, A.T., Hauser, A.W., Mathern, G., Moshé, S.L., Wiebe, S. and French, J., 2010. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 51, 1069-1077.

Lambrinoudaki, I., Kaparos, G., Armeni, E., Alexandrou, A., Damaskos, C., Logothetis, E., Creatsa, M., Antoniou, A., Kouskouni, E. and Triantafyllou, N., 2011. Bsml vitamin D receptor's polymorphism and bone mineral density in men and premenopausal women on long-term antiepileptic therapy. European Journal of Neurology 18, 93-98.

Lifshitz, F. and Maclaren, N., 1973. Vitamin D-dependent rickets in institutionalized, mentally retarded children receiving long-term anticonvulsant therapy. I. A survey of 288 patients. Journal of Pediatrics 83, 612-620.

Looker, A.C., Pfeiffer, C.M., Lacher, D.A., Schleicher, R.L., Picciano, M.F. and Yetley, E.A., 2008. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. American Journal of Clinical Nutrition 88, 1519-1527.

Mattson, R.H. and Gidal, B.E., 2004. Fractures, epilepsy, and antiepileptic drugs. Epilepsy & Behavior 5, S36-S40.

Menon, B. and Harinarayan, C.V., 2010. The effect of anti epileptic drug therapy on serum 25-hyrdoxyvitamin D and parameters of calcium and bone metabolism – a longitudinal study. Seizure 19, 153-158.

Mikati, M.A., Dib, B., Yamout, B., Sawaya, R., Rahi, A.C. and el-Hajj Fuleihan, G., 2006. Two randomized vitamin D trials in ambulatory patients on anticonvulsants: impact on bone. Neurology 67, 2005-2014.

Mintzer, S., Boppana, P., Toguri, J. and DeSantis, A., 2006. Vitamin D levels and bone turnover in epilepsy patients taking carbamazepine or oxcarbazepine. Epilepsia 47, 510-515.

Misra, A., Aggarwal, A., Singh, O. and Sharma, S., 2010. Effect of carbamazepine therapy on vitamin D and parathormone in epileptic children. Pediatric Neurology 43, 320-324.

Nettekoven, S., Strohle, A., Trunz, B., Wolters, M., Hoffmann, S., Horn, R., Steinert, M., Brabant, G., Lichtinghagen, R., Welkoborsky, H.-J., Tuxhorn, I. and Hahn, A., 2008. Effects of antiepileptic drug therapy on vitamin D status and biochemical markers of bone turnover in children with epilepsy. European Journal of Pediatrics 167, 1369-1377.

Nicolaidou, P., Georgouli, H., Kotsalis, H., Matsinos, Y., Papadopolou, A., Fretzayas, A., Syriopoulou, A., Krikos, X., Karantana, A. and Karpathios, T., 2006. Effects of anticonvulsant therapy on vitamin D status in children: prospective monitoring study. Journal of Child Neurology 21, 205-209.

O'Hare, J.A., Duggan, B., O'Driscoll, D. and Callaghan, N., 1980. Biochemical evidence for osteomalacia with carbamazepine therapy. Acta Neurologica Scandinavia 62, 282-286.

Offermann, G., Pinto, V. and Kruse, R., 1979. Antiepileptic drugs and vitamin D supplementation. Epilepsia 20, 3-15.

Pack, A.M., Morrell, M.J., Marcus, R., Holloway, L., Flaster, E., Done, S., Randall, A., Deale, C. and Shane, E., 2005. Bone mass and turnover in women with epilepsy on antiepileptic drug monotherapy. Annals of Neurology 57, 252-257.

Pack, A.M., Morrell, M.J., McMahon, D.J. and Shane, E., 2011. Normal vitamin D and low free estradiol levels in women on enzyme-inducing antiepileptic drugs. Epilepsy & Behavior 21, 453-458.

Pack, A.M., Morrell, M.J., Randall, A., McMahon, D.J. and Shane, E., 2008. Bone health in young women with epilepsy after one year of antiepileptic drug monotherapy. Neurology India 70, 1586-1593.

Pedrera, J.D., Canal, M.L., Carvajal, J., Postigo, S., Villa, L.F., Hernandez, E.R. and Rico, H., 2000. Influence of vitamin D administration on bone ultrasound measurements in patients on anticonvulsant therapy. European Journal of Clinical Investigation 30, 895-859.

Pluskiewicz, W. and Nowakowska, J., 1997. Bone status after long-term anticonvulsant therapy in epileptic patients: evaluation using quantitative ultrasound of calcaneus and phalanges. Ultrasound in Medicine and Biology 23, 553-558.

Reid, J.P., Von Muhlen, D., Miller, E.R., Michos, E. and Appel, L.J., 2009. Vitamin D status and cardiometabolis risk factors in the United States adolescent population. Pediatrics 124, e371-e379.

Russ, S.A., Larson, K. and Halfon, N., 2012. A National profile of childhood epilepsy and seizure disorders. Pediatrics 129, 256-264.

Saintonge, S., Band, H. and Gerber, L.M., 2009. Implications of a new definition of vitamin D deficiency in a multiracial US adolescent population: The National health and nutrition examination survey III. Pediatrics 123, 797-803.

Sato, Y., Kondo, I., Ishida, S., Motooka, H., Takayama, K., Tomita, Y., Maeda, H. and Satoh, K., 2001. Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy. Neurology 14, 445-449.

Shellhaas, R.A., Barks, A.K. and Joshi, S.M., 2010. Prevalence and risk factors for vitamin D insufficiency among children with epilepsy. Pediatric Neurology 42, 422-426.

Sheth, R.D., Wesolowski, C.A., Jacob, J.C., Penney, S., Hobbs, G.R., Riggs, J.E. and Bodensteiner, J.B., 1995. Effect of carbamazepine and valproate on bone mineral density. Journal of Pediatrics 127, 256-262.

Souverein, P.C., Webb, D.J., Weil, J.G., Van Staa, T.P. and Egberts, A.C.G., 2006. Use of antiepileptic drugs and risk of fractures: case-control study among patients with epilepsy. Neurology 66, 1318-1324.

Tekgul, H., Serdaroglu, A., Huseyinov, A. and Gokben, S., 2006. Bone mineral status in pediatric outpatients on antiepileptic drug monotherapy. Journal of Child Neurology 21, 411-414.

Valimaki, M.J., Tiihonen, M., Laitinen, K., Tahtela, R., Karkkainen, M., Lamberg-Allardt, C., Makela, P. and Tunninen, R., 1994. Bone mineral density measured by dual-energy x-ray absorptiometry and novel markers of bone formation and resorption in patients on antiepileptic drugs. Journal of Bone and Mineral Research 9, 631-637.

Valmadrid, C., Voorhees, C., Litt, B. and Schneyer, C.R., 2001. Practic patterns of neurologists regarding bone and mineral effects of antiepileptic drug therapy. Archives of Neurology 58, 1369-1374.

Verrotti, A., Greco, R., Latini, G., Morgese, G. and Chiarelli, F., 2002. Increased bone turnover in prepubertal, pubertal, and postpubertal patients receiving carbamazepine. Epilepsia 43, 1488-1492.

Volpe, S.L., Schall, J.I., Gallagher, P.R., Stallings, V.A. and Bergqvist, A.G.C., 2007. Nutrient intake of children with intractable epilepsy compared with healthy children. Journal of the American Dietetic Association 107, 1014-1018.

Wagner, C.L., Greer, F.R. and Nutrition, S.o.B.a.C.o., 2008. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 122, 1142-1152.

Wirrell, E., 2010. Vitamin D and bone health in children with epilepsy: fad or fact? Pediatric Neurology 42, 394-395.

Wong, J. and Wirrell, E., 2006. Physical activity in children/teens with epilepsy compared with that in their siblings without epilepsy. Epilepsia 47, 631-639.

Wong, T.S., Lau, V.M. and Fung, G., 2006. A survey of vitamin D in people with learning disability in long-stay hospital wards in Hong Kong. Journal of Intellectual Disability 10, 47-59.

Women and children

Key facts

- Despite good prenatal care, healthy maternal diets, and the availability of prenatal vitamins, gestational and neonatal vitamin D insufficiency is common.
- Global vitamin D insufficiency is a substantial concern affecting newborns regardless of geographic location, skin pigmentation, or socio-economic status.
- Contrary to the facts, many healthcare professionals and new parents believe that breast milk satisfies all their newborn infants' nutritional needs and that additional supplements, e.g. vitamin D, are not necessary.
- Vitamin D-related metabolites participate in the regulation of hundreds of genes and are utilized in every tissue in the body. The role of vitamin D begins early in fetal development and continues throughout life.
- Many experts believe that the adequacy of vitamin D in the neonatal period is essential to lowering the risk of certain chronic diseases like type 1 diabetes, cancer, and allergy.

Summary points

- Vitamin D adequacy in the newborn is totally dependent on the vitamin D status of the mother.
- Global insufficiency among all populations, including women of child-bearing age, means that large percentages of term infants and even larger percentages of preterm infants have inadequate supplies of vitamin D to support healthy development *in utero* and at birth.
- Breast milk is generally a poor source of vitamin D; infant formulas are much better sources but still may not supply enough vitamin D per serving to bring many infants to repletion within the first few weeks of life.
- Because vitamin D contributes to the regulation of so many genes in virtually every organ in the body (including those involved in cell cycle, differentiation, maturation and apoptosis), insufficiency early in life may have significant short and long-term negative health implications.
- Vitamin D insufficiency can adversely affect the ability of the neonate to ward off viral and bacterial infections and to properly develop natural immunotolerance. Conditions also linked to vitamin D inadequacy early in life include poor growth, asthma, allergy, bone disease, diabetes type 1 and 2, certain cancers, brain and mood disorders.
- Much more research is required to fully understand vitamin D in the neonate but there is a strong consensus that most infants should be provided with *at least* 400 IU vitamin D per day, beginning as early as possible after birth.

31. Neonatal effects of enhanced vitamin D and vitamin D for premature infants

H.K. Linke
Abbott Laboratories, Abbott Nutrition, Ross Park, 3300 Stelzer Road, Columbus, OH 43219, USA; hawley.linke@abbott.com

Abstract

Neonates must contend with the abrupt cessation in their supply of vitamin D and its metabolites. Due to widespread maternal insufficiency, the contemporary newborn has a high probability of suffering deficits for an indeterminate period in early life. Breast milk is a notoriously poor source of vitamin D and, while infant formula is fortified with the vitamin, the volume consumed in the neonatal period is insufficient to provide the recommended daily intake. The spread of insufficiency is global and may place infants at risk for short or long term deficits of growth, bone development, innate and adaptive immune responses, and increased risk of certain chronic diseases. Worldwide there is a range of recommendations regarding how much vitamin D is optimal during this stage and what doses of supplementation may be required. Regardless of advice, both healthcare professionals and families are often complicit in the failure to achieve repletion of vitamin D. The former frequently fails to adequately educate parents on the subject, while non-compliance is high among the latter. At this time, for most term infants, 400 IU/day starting as soon as possible after birth appears to be adequate. For preterm infants, even higher supplementation may be required in the neonatal period. Vitamin D plays a role in regulating at least 200 genes involved in growth, cell cycle control, differentiation, and maturation. These discoveries dictate that substantial research will be required to fully elucidate the demand for and significance of providing optimal cellular access to this pleiotropic secosteroid hormone during a critical window of development.

Keywords: recommendations, deficiency, supplementation, health outcomes

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_31, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyviatmin D
25(OH)D	25-hydroxyvitamin D
BMC	Bone mineral content
BMD	Bone mineral density
HIV	Human immunodeficiency virus
IL	Interleukin
NICU	Neonatal intensive care unit
PTH	Parathyroid hormone
OR	Odds ratio
RSV	Respiratory syncitial virus
SNP	Single nucleotide polymorphism
TLR	Toll-like receptor
T1D	Type 1 diabetes mellitus
UVB	Ultraviolet B
VDR	Vitamin D receptor

31.1 Introduction

With the snip of the umbilical cord an infant experiences a dramatic shift in physiologic dependence. While the most imminent need is to provide for their own respiration; they are also deprived of an immediate source of vitamin D and its metabolites. Subsequent nursing will not expeditiously restore the availability of this pleiotropic secosteroid hormone. In earlier eras humans evolved to harvest available sunlight for optimal cutaneous synthesis of vitamin D. The fetus would receive appropriate forms and amounts of vitamin D transplacentally and breast milk would provide adequate supplies to the newborn until they obtained access to sunlight to synthesize vitamin D for themselves. Circumstances which erode that process have developed over the previous few centuries. These include crowding in city environments, atmospheric pollution, global migration from sunlight-rich to sunlight-poor latitudes, cultural attitudes to cover exposed skin (primarily for women), time spent indoors at home and work environments, vegetarian diets, and, most recently, pressure to reduce skin cancer risk and photo-aging via chemical and physical sun screening.

These developments have provoked the well-documented emergence of vitamin D insufficiency at all stages of life and throughout the world. Newborn infants do not escape the effects of compromised vitamin D. Across latitudes and ethnicities, women of child-bearing age have a high prevalence of vitamin D insufficiency/deficiency which translates into low vitamin D levels in cord blood, neonates, and breast milk. (Balasubramanian and Ganesh, 2008; Basile *et al.*, 2007; Bassir *et al.*, 2001; Bodnar *et al.*, 2007; Bowyer *et al.*, 2009; Ginde *et al.*, 2009; Gordon *et al.*, 2008; Hatun *et al.*, 2005).

31.2 Neonatal vitamin D physiology

The neonatal period is dynamic in regard to vitamin D, calcium and PTH levels for the infant. Maternal vitamin D levels rise during pregnancy; in the placenta both 25(OH)D and $1,25(OH)_2D$ are available; and conversion of 25(OH)D to the active hormone occurs in maternal tissues, decidual and placental tissue, as well as the fetal kidney. Calcium stores are also enhanced in the period before delivery and PTH levels are generally low (Salle *et al.*, 2000). Calcium plasma concentrations decrease right after birth and PTH levels correspondingly rise, but both return to normal by the second week. The transient PTH elevation stimulates synthesis of $1,25(OH)_2D$ in the newborn. Higher levels of maternal vitamin D tend to subdue these perinatal fluctuations and help maintain better vitamin D status (Salle *et al.*, 2000). In preterm infants, assuming adequate precursor, $1,25(OH)_2D$ levels increase in the first month and are well maintained through the first 3 months. Alternatively, preterm vitamin D deficiencies at this point contribute to hypocalcemia which is a major factor in osteopenia associated with prematurity (Bishop *et al.*, 2005).

Many reviews point to the 1940's as the era during which vitamin D-deficient rickets was virtually eliminated in the United States, primarily due to access to vitamin D fortified milk (e.g. Holick, 2006). By 1979, however, an 'outbreak of rickets' in urban Philadelphia (Bachrach *et al.*, 1979) served as a harbinger for the deluge of similar reports. Over the next two decades reports of rickets and vitamin D deficiency extended this limited observation to a variety of populations, even, unexpectedly, to light-skinned breastfed infants in Iowa (Ziegler *et al.*, 2006). Although the American Academy of Pediatrics, aware of the limited vitamin D available in most breast milk, had long recommended 400 IU/day vitamin D supplementation, several studies document both a failure of health care providers to recommend supplementation, and, if recommended, parental resistance to compliance (Garganta and Buchana, 2012; Huurre *et al.*, 2006; Kreiter *et al.*, 2000; Ling, *et al.*, 2011; Merewood *et al.*, 2009; Perrine *et al.*, 2010; Taylor *et al.*, 2010; Williamson and Greene, 2007).

A 2010 compilation of reports, (Dror and Allen, 2010), highlights global vitamin D status during pregnancy and includes data on inadequacies in cord blood. Eight reports between 2005-2009 from India, Iran, Australia, USA, Greece and Canada described cord blood insufficiency/deficiency for infants of diverse ethnicity. The brightest spots in the data were the relatively low percent of deficiency in Athens, Greece (8.1%), Sydney, Australia (11%) and the moderately low level in Winnipeg, Canada (36%). All the other populations, however, displayed cord blood deficiency from 55% (Boston, USA) to 96% (Lucknow, India).

In these and similar reports the cut-off level assigned for maternal and cord blood insufficiency and deficiency varied: deficiency <25 nmol/l to <37.5 nmol/l; insufficiency <50 nmol/l (in all but one study which invoked <80 nmol/l). Disturbingly, a large study of vitamin D supplementation (400, 2,000 or 4,000 IU/day) for pregnant women, suggests an even higher standard should be assigned for sufficiency. Hollis *et al.* (2011) established that during pregnancy conversion of 25(OH)D to $1,25(OH)_2D$ (the biologically active form) was not optimized until plasma levels of the former reach 100 nmol/l. Among the specific complications of pregnancy that were

documented, preterm labor, preterm birth and infection were inversely related to 25(OH)D. Complications were lowest in the 4,000 IU/day group for which the risk of these comorbidities was reduced by 50%. Interestingly, preterm labor and birth were also inversely correlated to vitamin D status at baseline (12-14 weeks gestation). There were no adverse events associated with the vitamin D treatments (Wagner *et al.*, 2010, 2012). Whether this daily dose should be extended for the infant and/or the mother postnatally and whether it confers long-term benefit to the newborn remains to be determined.

While adiposity, veiling and dark skin contribute to some of the higher incidences of inadequacy, unveiled and lighter skin populations also add to the pool of deficient cord blood values (Dijkstra *et al.*, 2007; Dror and Allen, 2010). The general pattern of deficiency/insufficient prevails globally, particularly among breast fed infants (Challa, *et al.*, 2005; Dawodu *et al.*, 2010; Ziegler, *et al.*, 2006). While many advisory bodies recommend vitamin D supplementation for newborns (milk and formula fed), compliance falls short of expectations.

31.3 Vitamin D related health outcomes for infant

31.3.1 Bone, calcium and other classic vitamin D actions

The primary benefit of vitamin D for infant bone health and calcium homeostasis has been established for nearly a century (Holick, 2006). In fact, some current professional recommendations for vitamin D are based solely on maintenance of infant-child skeletal health. This perspective is reviewed in some detail in the 2011 IOM North American report providing vitamin D recommendations (Ross *et al.*, 2011). Concurrent with unraveling the role of vitamin D receptors, vitamin D response elements, and gene regulation by vitamin D, in virtually every tissue type in the body (Kimball *et al.*, 2008), the resurgence of rickets in otherwise healthy newborns spurred renewed attention to vitamin D. Because the role of vitamin D (and calcium/phosphorous) in bone health of infants has been so extensively addressed by others – from the molecular to the clinical level for both preterm and term – it is not the focus of this review. It is sufficient to note that the infant displaying 25(OH)D levels below 25 nmol/l is at risk for rickets or suboptimal bone mineral content or bone mineral density. The presence of adequate calcium supplementation plus ≥400 IU/day vitamin D and/or UVB exposure will mitigate risk and restore bone health in the otherwise uncomplicated condition (Gallo *et al.*, 2010; Pehlivan, 2003).

However, vitamin D supplementation during infancy may have longer lasting skeletal effects. In one study bone status was obtained from 106 8-year-old Caucasian girls (formerly breastfed) with or without vitamin D (400 IU/day) during their first year. The prepubertal analysis associated infancy supplementation with higher BMC and areal BMD at multiple bone sites (Zamora, 1999).

31.3.2 Beyond bone: pleiotropic effects of vitamin D

The proliferation of vitamin D research in the neonatal period reflects an expanding interest in the non-traditional, non-calcemic, non-bone related pleiotrophic actions of $1,25(OH)_2D$. Activation of 25(OH)D to $1,25(OH)_2D$ occurs primarily in the kidney, but cell types throughout the body express the enzymes necessary for local conversion (e.g. 1-αhydroxylase). Starting early in development, these and other cells also express nuclear vitamin D receptors. Binding to the VDR can trigger a rapid cellular signaling response that modifies activities like absorption of calcium by the intestine and secretion of insulin by β cells. It can also follow a path to regulate the transcription of genes via vitamin D response elements on DNA. In this way the active hormone has been shown to modulate the expression of over 200 mammalian genes (Goldring *et al.*, 2011; Taylor *et al.*, 2009). Therefore vitamin D, its derivatives, and their physiologic accessibility have the capacity to impact each tissue in the body.

Many of the attributes of adequate vitamin D demonstrated in other age groups are likely to apply in neonatal life. Over time, inspired by significant animal and *in vitro* discoveries, results from randomized placebo-controlled and prospective observational trials are being reported along with epidemiologic studies. The sections below describe a few non-calcemic benefits related to the neonatal stage.

31.3.3 Securing achievement of growth potential

In a 1968 retrospective symposium, Genevieve Stearns, an infant feeding pioneer, described historic experiments evaluating the impact of exogenous vitamin D on the growth of infants fed dilute condensed milk, soured whole milk or similar breast milk substitutes common in the 1930s. Researchers concluded that approximately 300-800 USP units (IU) daily provided an optimal trajectory of linear growth with earlier dentition and 'precocious muscular achievements'. They were chastised for creating infant 'monsters' until subsequent work verified similar growth advantages for breast fed infants similarly supplemented (standardized cod liver oil or milk from specially fed cows) (Stearns, 1968). While feeding practices and formula compositions have evolved in the subsequent 50+ years, adequate vitamin D continues its role facilitating normal growth in both term and preterm infants.

A review of lifetime health records from the Northern Finland Birth Cohort 1966 focused on recorded estimates of vitamin D supplementation during year one. Heights were compared for nearly 10,000 subjects at three time points. At one year, height of infants without supplementation appeared to lag slightly behind those receiving any supplementation (P=0.005), but the significance evaporated after adjustments for multiple factors. At age 31, however, subjects who experienced rickets in infancy – regardless of compensatory treatment and other adjustments – were ~0.5 cm shorter than others (P=0.01). This latter finding suggests that early deficiency can have permanent growth consequences (Hypponen, 2011).

Among preterm infant vitamin D supplementation trials, however, studies did not observe differences in either short term growth parameters (during supplementation) or long-term (at 9-11 years of age) when comparing two higher levels of vitamin D supplementation (400-500 IU/day vs. ~1000 IU/day) (Backstrom *et al.*, 1999a,b; Fewtrell *et al.*, 2000).

The general conclusion from many growth studies is that while vitamin D supplementation is prudent for preterm, breastfed, and formula fed infants, daily doses exceeding 400-500 IU are probably not required for normal growth outcomes in healthy infants.

Nonetheless, certain neonatal conditions demand greater attention to circulating vitamin D levels. In New Delhi, India, among a vulnerable population, Kumar followed LBW term infants supplemented with vitamin D (1,400 IU/week) over the first six months. Supplementation provided better vitamin D status at six months and significantly increased standard deviation *z*-scores for weight, length, and arm circumference, and decreased the proportion of children with stunted growth (length for age *z*-score ≤2) or with arm circumference *z*-scores of 2 or less (Kumar, 2011).

In a controlled study of vitamin D supplementation among third trimester pregnant Asian women living in London (presumed to represent low vitamin status). Maxwell *et al.* (1981) found no differences in infant weight and length at birth, three, or six months of age. From six to 12 months, however, both weight and length diverged, producing an 8% advantage in weight and a 2% advantage in height for the infants of supplemented women.

Infants born with vitamin D insufficiency and who remain at high environmental risk for nutrition-dependent rickets frequently experience growth rate reduction. Forty-six such infants (mean age 13 months) were compared with 40 normal matched controls before, and six months after, vitamin D treatment. Treatment was accompanied by a growth spurt which appeared at the time to adequately compensate most infants for the growth retardation experienced during deficiency. Biochemical measurements during recovery identified significant correlative increases in IGF-1. The authors speculate that the IGF-1 reduction observed during vitamin D deficiency is a compensatory response designed to slow growth during a period of calcium insufficiency (Soliman *et al.*, 2008).

31.3.4 Infection/immunity/allergy/asthma

Important roles for vitamin D in the immune system have been well established for most segments of the population and the perinatal period is no exception. To test one measure of the adaptive immune system, cord blood levels of vitamin D from 101 term Turkish infants were evaluated against the numbers of regulatory and helper T cells. While investigators failed to identify a relationship between T cell counts and vitamin D concentration, they noted that one-third of the blood samples exhibited <12ng/ml 25(OH)D (very deficient), while only 7% of bloods registered >25 ng/ml (sufficient). This high incidence of insufficiency appeared to have

directly affected the T cells' overall ability to respond physiologically, thus precluding detection of putative modulation of T cell activity (Guven *et al.*, 2012).

Since infants' T and B cells are immature, neonates are more dependent on innate immune responses over adaptive responses to resist early infection. *Ex vivo* techniques have been used to examine innate immune response at birth. Twenty-three cord blood plasmas from a predominantly Hispanic population in Southern California were measured for 25(OH)D and $1,25(OH)_2D$ concentrations. Unlike adult vitamin D plasma relationships, concentrations of these two molecules were highly correlated in cord blood. Based on 25(OH)D analyses, 17% of neonates were identified as severely deficient (<12 ng/ml); 22% were sufficient (>30 ng/ml) and the remaining 61% were distributed between insufficient and deficient categories. In *ex vivo* studies of human monocytes, severely deficient cord plasma, compared to sufficient plasma, was significantly less supportive of innate immunity responses normally invoked to protect against both gram-negative (TLR4-mediated) and gram-positive (TLR2-mediated) infections. Fortification of deficient plasma with either form of vitamin D significantly restored both TLR4 and TLR2 related antimicrobial pathway gene expression (Walker *et al.*, 2011). Also using *ex vivo* technique, Hirsch *et al.* (2011) examined the anti-inflammatory responses regulated by $1,25(OH)_2D$ concentrations of neonatal neutrophils from cord blood (collected in northeast USA). They concluded that exogenous $1,25(OH)_2D$ was far less active in suppressing markers of inflammation in the neonatal immune cells compared to normal adult cells. The lessened sensitivity may have been the result of the observed low expression of both VDR and 1-αhydroxylase in the neonatal cells. They cautioned that inherent insensitivity of neonatal immune cells to vitamin D regulation raises further concerns regarding worsening evidence of insufficiency in newborns. That is, if neonatal immune cells are physiologically compromised during *in utero* insufficiency, then postnatal levels must be even higher to support proper vitamin D regulation of immune maturation.

Other studies directly connect vitamin D to perinatal immune health outcomes., e.g. cord blood vitamin D deficiency seems associated with RSV bronchiolitis. In their first year, Dutch neonates born with 25(OH)D levels <50 nmol/l had a six times greater risk of RSV bronchiolitis than those with ≥75 nmol/l (Belderbos, 2011). This longitudinal study supports several earlier studies linking vitamin D to RSV, rhinovirus, and other viral infections.

The relationship of perinatal vitamin D to mother-child transmission of HIV is also significant. Adult studies suggest that low vitamin D levels may be associated with negative outcomes for HIV infected patients (e.g. Viard, 2011). In a pregnancy study, vitamin D levels were obtained from 884 HIV positive pregnant Tanzanian women in a multiple vitamin plus D supplementation trial. There was no association between vitamin D and poor pregnancy outcomes including low birth weight and preterm birth. However, newborns from mothers with <32 ng/ml vitamin D were at significantly greater risk for HIV at 6 weeks, HIV transmission via breast milk, and overall risk of HIV infection (46% higher risk). Those infants also had a 61% higher risk of dying during the first two years (Mehta *et al.*, 2009). As suggested in subsequent correspondence, vitamin D may be acting through both the peripheral immune system and on the placental ability to modulate

infection (Equils and Hewison, 2010). The need for more rigorous evaluation of vitamin D in the prevention of HIV transmission to newborns is apparent.

Intervention trials of vitamin D supplementation and protection from infant infections may be sensitive to how much, when and to whom the intervention is applied. As reviewed recently by Yamshchikov *et al.* (2009), evidence from predominantly adult clinical trials suggests sufficient support to further investigation into vitamin D therapy for tuberculosis, influenza, viral upper respiratory tract infections, and all-cause infection rates. A double blind trial of placebo and vitamin D conducted among 3,000 high risk infants (1-11 months old) in Kabul appears to be interestingly inconsistent. 100,000 IU boluses of vitamin D3 were delivered every three months and incidence and severity of pneumonia was evaluated during every-two-week visits by fieldworkers. This supplementation regimen did not affect the incidence or severity of pneumonia, hospital admissions, or all-cause mortality. Interpretation of this result from such a large study is complicated by many factors including the appropriateness of 'bolus' delivery, with its own unknown consequences related to oscillating vitamin D resources for infants; the (mal)nutritional background and general high-risk nature of the population; and the lack of identification of etiologic agents or human genotypes in regard to vitamin D (Manaseki-Holland *et al.*, 2012). While this report is useful for identifying the inefficacy of this regimen, future studies will necessitate more complex variations to identify specific neonatal immune effects of adequate vitamin D currently predicted from *in vitro*, animal and correlative studies.

An evaluation of genetics and vitamin D concentration in cord blood revealed a combined effect on food sensitivity of infants around two years of age. In Boston, 649 children enrolled at birth were tested for IgE sensitivity to common food allergens. Toddlers were compared on the basis of cord blood vitamin D, food sensitivity, and polymorphisms (SNP) around 11 genes important for IgE and vitamin D regulation. Cord blood vitamin D deficiency was more prevalent in food sensitive toddlers than control (P=0.09). Additionally an SNP for IL4 expression, when combined with cord blood deficiency, created an increase risk for sensitization. (OR=1.79). When further combined with three SNP involving other genes, a strong gene-deficiency-sensitivity interaction was observed ($P_{\text{interaction}}=9\times10^{-6}$) (Liu *et al.*, 2011).

Independent of specific immune outcomes, the genome-wide association study (34,000 individuals of European descent) by Wang *et al.* (2010) further describes at least three genome variants that identify subjects at increased risk for simply maintaining 25(OH)D concentrations <75 nmol/l (OR=2.47) or <50 nmol/l (OR=1.92) Others have indicated difference in kinetics around vitamin D synthesis and actions between racial/ethnic groups (Dawson-Hughes, 2004; Gutierrez *et al.*, 2012; Signorello *et al.*, 2011; Sweeney *et al.*, 2006). Thus, our ability to come to meaningful conclusions about the role of vitamin D and the immune system in the neonatal period may require attention to details regarding race, genetics, and environment.

Heart failure in infants

Hypocalcaemia can induce cardiac effects in adults and occasional reports of cardiomyopathy associated with rickets in infancy and childhood have been described. A review of patient records in England identified 16 infants (3 weeks to 8 months old; black or Indian ethnicity) with dilated cardiomyopathy associated with vitamin D deficiency. The infants had very low levels of calcium, high PTH and 25(OH)D median 18.5 nmol/l (0.00 to 43.00). Ten infants had radiologic evidence of rickets. This association is especially interesting given that no white infants were identified under these criteria over the six year review period (Maiya *et al.*, 2007).

From a different perspective Shedeed (2012) hypothesized that the impact of proinflammatory cytokines observed in congestive heart failure might be abated by vitamin D intervention. Eighty infants with congestive heart failure were randomized to placebo or 100 IU/day cholecalciferol. All were treated for 12 weeks with standard antifailure regimen. All vitamin D concentrations were low at enrollment. As anticipated, both groups responded with improvement in heart failure scores. But significantly greater improvement of the vitamin D treatment group over the placebo group was identified for heart failure score, left ventricular end-diastolic dimension, left ventricular end-systolic dimension, ejection fraction, early and late ventricular filling velocity ratio, myocardial performance index, PTH (reduced), IL10, IL6 (reduced), and tumor necrosis factor-α (reduced). Mean 25(OH)D concentration in placebo subjects was 14.64 ng/ml and supplemented infants 32.89 ng/ml. The author concluded that vitamin D supplementation not only served as an anti-inflammatory agent in infants with congestive heart failure but helped to accelerate clinical improvement and cytokine balance.

31.3.5 Type 1 diabetes

Current knowledge about the relationship between vitamin D and the development of the autoimmune disease T1D engenders a complex web that involves geography, autoimmunity, polymorphism of vitamin D-related genes, effects on insulin usage, supplementation regimen, and circulating vitamin D levels. Vitamin D supplementation during the neonatal/infancy period was first implicated as protective against T1D in a 1999 report from the EURODIAB ACE Study Group (Anonymous, 1999). Later a meta-analysis of five observational studies further supported significantly reduced, possible timing- and dose-dependent risk for T1D with vitamin D supplementation in infancy (Zipitis and Akobeng, 2008). There is also a striking relationship between increasing latitude and increasing T1D risk (Hypponen *et al.*, 2001, 2010), or month of birth (Kahn *et al.*, 2009) primarily attributed to decreased UVβ irradiation potential early in life. Correlative observations from a variety of locations (Switzerland to Saudi Arabia) (Bin-Abbas *et al.*, 2011; Janner *et al.*, 2010) continue to show vitamin D deficiency among T1D children. Other association studies link certain vitamin D metabolism gene variants with T1D susceptibility (Bailey *et al.*, 2007).

Two recent reports prospectively studying 'at risk' populations during infancy have not supported either vitamin D levels at 9 months of age or maternal intake as protective for T1D. It should be

noted, however, these are genetically selected populations, vitamin D at 9 months is a single static measurement past the neonatal period, and (in the first study) risk was determined based on a biomarker of islet immunity (Simpson *et al.*, 2011). The second study is predicated only on self-reported maternal intake, without analysis of resulting vitamin D concentration in mothers or their infants (Marjamaki *et al.*, 2010). Justification of the correlative observational results (above) with these discordant prospective studies remains to be elucidated.

31.4 Special circumstances for preterm and small for gestational age infants

The prematurely born infant faces many specific developmental challenges which may be exacerbated by low availability of vitamin D. This section refers to those born <37 weeks and LBW (<2,500 grams), VLBW (<1,500 grams), and ELBW (<1000 grams). Unperturbed development of immature tissues and systems is at risk when *in utero* vitamin D supply is prematurely terminated. Maintaining an adequate supply of nutrients – many of which are normally enhanced during the final weeks of human gestation – is extraordinarily difficult in the preterm infant especially one who has also experienced deficits *in utero*.

There is currently no consensus regarding the optimal level of vitamin D supplementation for preterm infants. While the IOM (Ross *et al.*, 2011) concluded that investigation has 'failed to show clinical value above 400 IU/d in preterm' Backstrom (1999) and Koo *et al.* (1995) stated '160 IU/d is adequate' based on a study with 20-21 neonates per group. This latter perspective is not in current favor among many experts (Stephens *et al.*, 2009). Among other issues, despite randomization, the groups in the Koo study were not equivalent in ethnicity, height, weight, weight gain or duration of feeding.

Newer studies of vitamin D levels and supplementation strategies have come to light regarding preterm and/or infants with low birth weights. Most of these studies were conducted in the United States. While the protocols were variable, they combine to provide an important narrative. Prevalence of deficiency (<20 ng/ml) at birth was high, i.e. ~50% to nearly 90%, regardless of race, (although African Americans > Hispanics > Caucasians) or gestational age (although less mature > more mature). Standard vitamin D supplementation procedures were successful at raising plasma concentrations but not necessarily before discharge from the NICU. Frequently infants did not receive the recommended 400 IU/day from nutritional intake for many weeks after birth. Only specific supplement dosing from daily drops or IV administration resulted in rapid recovery from deficiency acquired *in utero* (Cifuentes *et al.*, 2012; Hanson *et al.*, 2011; Monangi *et al.*, 2012; Munshi *et al.*, 2012; Porcelli *et al.*, 2012; Taylor *et al.*, 2012a,b; Viswanathan *et al.*, 2012). Data from preterm infants in the United Arab Emirates yielded similar conclusions except that deficiency was more severe, with 44% of infants at risk for metabolic bone disease due to vitamin D <12.5 nmol/l (Dawodu and Nath, 2011).

The building consensus is that the pleiotrophic responsibilities ascribed to vitamin D may not be satisfied by the vitamin D level shown to be adequate for bone development (>20 ng/ml) (Holick *et*

al., 2012). Some experts advocate that newer calcium-related data support a sufficiency minimum of 30 ng/ml. While others recommend the concentration at which fluctuation in PTH stabilizes (Holick *et al.*, 2012; Tylavsky *et al.*, 2005). Giapros *et al.* (2012) examined PTH and vitamin D levels of 128 preterm (32-36 weeks) Greek infants. During at least the first three months most infants were vitamin D insufficient (<30 ng/ml) and many displayed elevated PTH. The inverse relationship and PTH stabilization threshold in this group was used to justify a reasonable cutoff point for vitamin D insufficiency in late preterm infants as 32 ng/ml (Giapros, *et al.*, 2012).

Many of the vitamin D-related morbidity concerns for preterm infants will be similar, if not exaggerated, versions of the concerns identified for term infants. Logically, the preterm infant's attempt to continue *in utero* programs of proliferation, differentiation and maturation in most cell and organ types, adds to the necessity of adequate vitamin D for appropriate regulation. Its role in inflammation and immunity will also be important for the vulnerable preterm infant. The full implications, specifically during human gestation and premature birth outcomes, are still under investigation (Golding *et al.*, 2011). One example of preterm sensitivity is osteopenia of prematurity or metabolic bone disease. Reduced bone mineral content, possibly with rachitic changes and even bone fracture is the result of inadequate vitamin D, calcium and phosphorous with accompanying inactivity and use of certain drug therapies in the NICU (Bozzetti and Tagliabue, 2009). Adequate vitamin D is definitely important, but many clinicians also suggest that the adequacy of other components are primary to the disorder prevention and treatment (Lam *et al.*, 2007). In the lung, rachitic respiratory distress also occurs in early preterm infants (Goldring *et al.*, 2011).

These and similar concerns suggest that vitamin D should be routinely evaluated in preterm infants and that strategies for repletion should reflect vitamin D dosing assuring at least 400 IU/day; more as individual circumstances might demand. The specifics of strategies and targeted plasma levels will benefit from further optimization in clinical evaluation. Adequately powered studies targeting intervention for short- and long-term health outcomes in this population have not yet been conducted.

31.5 Recommendations for vitamin D in the perinatal period are highly variable

Health agencies around the world debate assignment of the ideal plasma concentration of vitamin D in term infants, the speed with which or age by which a neonate should attain that level, and the amount of supplementation required to sustain optimal plasma concentration. Opinions, even those based on the same studies, vary depending on the value placed on bone- versus non-bone-related outcomes; the populations studied versus the populations served; value given to molecular inferences for long-term benefits versus health outcomes demonstrated in small, short-term trials; or whether caution is best applied in the absence of compelling extra skeletal benefit or based on the previously demonstrated safety profiles of higher daily doses (Fischer *et al.*, 2008; Henderson, 2005; Holick *et al.*, 2006; Holmlund-Suila *et al.*, 2012; Onwuneme *et*

al., 2012; Pludowski *et al.*, 2010; Ponnapakkam, 2010; Saadi *et al.*, 2009; Siafarikas *et al.*, 2011; Trussler *et al.*, 2008a,b; Will *et al.*, 2009).

31.5.1 Europe – ESPGHAN

A 2010 recommendation from the ESPGHAN Committee on Nutrition discusses the implications of newer data and broader requirement (non-calcemic) for vitamin D in plasma. Preterm infants specifically, may require 800-1500 IU per day to reach a plasma level >80 nmol/l (Agostoni *et al.*, 2010). The ESPGHAN recommendation for term infants established in 2005 remains at 40-100 IU/100 kcal for infant formula and 40-120 IU/100 kcal for follow-on formula (Kotelzko *et al.*, 2005; Scientific Committee on Food, 2003).

31.5.2 Europe – France

The Committee on Nutrition of the French Society of Paediatrics has provided the most recent opinion on this subject. For many decades specific infant supplementation ranged from 1000 to 2,500 IU/day depending on infant characteristics. In the 1990's infant formula was also fortified (40-100 IU/100 kcal). France has continued to stress the need for all breastfed infants to receive supplemental vitamin D of 1000 IU/day and specifically attribute these higher guidelines for preventing the resurgence of rickets seen elsewhere in Europe and North America (Vidailhet *et al.*, 2012).

31.5.3 Australia and New Zealand

This part of the world traditionally relied on the abundance of sunlight, weighing minimum exposure for vitamin D adequacy against skin cancer risk (Stalgis-Bilinski *et al.*, 2011). While detailed discoveries regarding specific infant populations continue to develop in this region, the recommendation adapts guidelines from elsewhere, e.g. pregnant women should be evaluated for sufficiency in the first trimester and, if deficient, should receive 3,000-5,000 IU/day until serum levels are above 50 nmol/l. At that point they join other women at 400 IU/day supplementation. Infant formulas are fortified at 400 IU/l, but all infants breastfed or others not consuming at least 500 ml/per day formula should be supplemented with 400 IU/day vitamin D (Diamond *et al.*, 2005; Munns *et al.*, 2006).

31.5.4 North America

In North America, the Institute of Medicine, revisiting vitamin D requirements, concluded that compelling data for setting guidelines only existed for calcium/bone-related outcomes. It also rejected the perspective that vitamin D insufficiency is a substantial concern in the population and found no justification for routinely evaluating vitamin D circulation even in preterm infants (Term infant recommendation = AI 400 IU for 0-12 months, UL 1000 IU 0-6 and 1,500 IU 6-12 months). Their recommendation targets desirable infant blood level as 40-50 nmol/l. The document presumes practically all persons are sufficient at 50 nmol (Ross *et al.*, 2011).

Most experts agree that 400 IU/day is likely adequate for maintaining sufficiency in the first year of life. To date, there have been no intervention studies that propose an appropriate timetable for attaining adequate plasma vitamin D, given the high incidence of *in utero* and perinatal insufficiency. The IOM report suggests that routine screening is inappropriate except for populations at high risk. However, Kersey *et al.*, (2011) point out that low income children in the United States are routinely screened for anemia and blood lead levels. The prevalence of those potentially damaging diagnoses in the population studied were 10% and 4% respectively. In the same group, however, vitamin D deficiency (<20 ng/ml) or insufficiency (<30 ng/ml) were 29% and 62% respectively. The comparison makes a strong case for modification of current policy on screening activities.

The AAP preterm recommendation from 2008 aligns with the IOM recommendation of maintaining the minimum 400 IU/ day in this population. However, AAP proposes value in monitoring vitamin D status of the preterm, targeting a goal >50 nmol and finds significant clinical evidence of safety allowing provision of 400 to 1000 IU per day with milk fortifier, preterm formula and/or a vitamin preparation. The preterm infant is likely to be at greater risk for deficiency at birth and less likely to receive adequate supplementation in early days when internal feeding is limited (Groh-Wargo and Sapsford, 2009).

In 2010 The Canadian Pediatric Society confirmed their 2007 recommendation of 400 IU/day supplementation for term infants to prevent severe vitamin D deficiency (<25 nmol/l). They note, however, that new data suggests that optimal vitamin D status may require plasma concentration of >75 nmol/l; for which the recommended intake will not be sufficient. They also acknowledge that First Nations populations and others with darker skin or living in far northern areas may require additional supplementation especially during winter (First Nations, Inuit and Métis Health Committee, 2012).

31.6 Making recommendations work

Researchers at the Centers for Disease Control and Prevention in the United States analyzed vitamin D intake data during the first 10.5 months. Intakes were evaluated against both 2003 and 2008 AAP recommendations (200 and 400 IU/day respectively). Regardless of feeding type, vitamin D supplementation was actually rare over the period studied: ≤4% of formula fed infants; ≤12.6% of breast milk fed infants. While most formula fed met the 2003 recommendation, only 20 to 37% matched the 2008 values. Among the breast milk fed, the percent of infants who met either the 2003 or 2008 standards was predictably identical to the percent who actually received vitamin D supplements. Although formula contains ~400 IU/l vitamin D, this study found that many infants do not consume a full liter per day, which can explain the failure of so many to meet the 2008 standard of 400 IU/day (Perrine *et al.*, 2010).

In Europe, North America, and other regions of the world, vitamin D liquid supplements (vitamin D alone, in multivitamins for infants, or in natural preparations like cod liver oil) are available

without charge or at low cost through health agencies. Nonetheless, a variety of surveys and assessments address why many infants with easy access are not receiving adequate vitamin D. Even among US military physicians only ~70% reported in 2007 recommending supplements for breastfed infants and ~48% recommended vitamin D for formula fed infants. The most common reasons for not recommending were the beliefs that there was adequate sunlight exposure and that breast milk contained adequate vitamin D. None reported taste, cost, or risk of hypervitaminosis as contributing factors (Sherman and Svec, 2009). Encouragingly, these levels are a substantial improvement over reports published just 5 years earlier regarding the civilian populations in the sunny cities of Las Vegas and San Diego (Hayward *et al.*, 1987; Shaikh and Alpert, 2004).

In some regions where sunlight is less abundant, physicians and parents seem better educated about the need for vitamin D supplementation. In Montreal, Canada, e.g. 74% of mothers exclusively breast feeding in 2008 reported meeting the Health Canada supplementation recommendation, up from 58% from a study reported in 2004 (Lepage *et al.*, 2004). However supplementation still declined significantly as infants became partially or exclusively formula fed. Nearly 600 mothers of 2 month old infants in British Columbia, Canada were surveyed in 2010 for compliance with health care professional recommendations. 91% of exclusively breastfeeding mothers reported supplementation with vitamin D as did 20% of exclusively formula feeding mothers. Mixed feeding infants fell between these groups. Over 60% of infants received 300-500 IU vitamin D/day, while only 5% reported no oral vitamin D. (Crocker BMC Public Health, 2011). While many rely on formula to provide sufficient vitamin D, it is not always adequate at the volumes consumed (Gallo *et al.*, 2010). The inadequacy in formula vitamin D fortification is due in part to manufacturers' limitations and guidance currently provided by government agency guidelines or laws (see Infant Formula Act of 1980) (Infant Formula Act, USA, 1980; Newberry, 1982).

Several groups have investigated means to further support parents' initiation and maintenance of neonatal vitamin D supplementation, particularly among the highest risk populations. In Norway, in 2004, the standard care was to offer mothers relevant information and encouragement to deliver vitamin D supplements to their infants. An intervention study demonstrated that providing a brochure, available in languages of those at highest risk, and a free seven week supply of vitamin D2 drops, produced a 28 nmol/l improvement in vitamin D status when compared to standard of care. The protocol was especially useful for breast fed infants (Madar *et al.*, 2009). Studies of palatability of vitamin D vehicles (oil vs. alcohol) (Pronzini *et al.*, 2008) and ease of administration (filmstrip vs. dropper) (Rodd *et al.*, 2011) identify means to substantially improve compliance. Vitamin D drops that are more concentrated (i.e. 400 IU/drop vs. 400 IU/ml) have the added advantage that applying a drop to a pacifier or the mother's breast is a convenient form of delivery (Casey *et al.*, 2010). It should be noted that attempting to fortify breast milk with maternal supplements is an inefficient process. Some reports indicate maternal ingestion of 6,400 IU/day may be necessary to increase vitamin D in breast milk such that the mother provides her infant sufficient vitamin D roughly equivalent to that of infants directly supplemented with 400 IU/day (Wagner *et al.*, 2006).

The information available regarding both counterproductive and effective measures for supplementation should be considered whenever health agencies undertake efforts to improve vitamin D status of newborns.

31.7 Conclusion

Maintaining vitamin D adequacy in the perinatal period and beyond has the potential to positively affect human health well beyond its indisputable role in mineral regulation and bone support. As the non-traditional metabolic activities regulated by availability of vitamin D-related molecules become better understood, there will also emerge an appreciation for the temporal constraints around vitamin D benefits and risks. The neonatal period – characterized by rapid growth, substantial development, and even epigenetic modifications and programming – may prove to be a linchpin for a lifetime of health consequences or benefits. As such it must remain a prime target for research funding, well-designed clinical trials, and open-minded, ongoing evaluation by those entrusted with human health decisions and policy.

References

Agostoni, C., Buonocore, G., Carnielli, V.P., De Curtis, M., Darmaun, D., Decsi, T., Domellöf, M., Embleton, N.D., Fusch, C., Genzel-Boroviczeny, O., Goulet, O., Kalhan, S.C., Kolacek, S., Koletzko, B., Lapillonne, A., Mihatsch, W., Moreno, L., Neu, J., Poindexter, B., Puntis, J., Putet, G., Rigo, J., Riskin, A., Salle, B., Sauer, P., Shamir, R., Szajewska, H., Thureen, P., Turck, D., Van Goudoever, J.B. and Ziegler, E.E.; ESPGHAN Committee on Nutrition, 2010. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. Journal Pediatric Gastroenterology and Nutrition 50(1), 85-91.

Anonymous, 1999. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. Diabetologia 42(1), 51-54.

Anonymous, Guidance & Regulatory Information, Infant Formula Act. Available at: http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/InfantFormula/GuidanceRegulatoryInformation/default.htm.

Bachrach, S., Fisher, J. and Parks, J.S., 1979. An outbreak of vitamin D deficiency rickets in a susceptible population. Pediatrics 64(6), 871-877.

Backström, M.C., Mäki, R, Kuusela, A.-L., Sievänen, H., Koivisto, A.-M., Ikonen, R. S., Kouri, T. and Mäki, M., 1999b. Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants. Archives of Disease in Childhood. Fetal and Neonatal Edition 80, F161-F166.

Backström, M.C., Mäki, R., Kuusela, A.L., Sievänen, H., Koivisto, A.M., Koskinen, M., Ikonen, R.S. and Mäki, M., 1999a. The long-term effect of early mineral, vitamin D, and breast milk intake on bone mineral status in 9- to 11-year-old children born prematurely. Journal of Pediatric Gastroenterology and Nutrition. 29(5), 575-582.

Bailey, R., Cooper, J.D., Zeitels, L., Smyth, D.J., Yang, J.H., Walker, N.M., Hyppönen, E., Dunger, D.B., Ramos-Lopez, E., Badenhoop, K., Nejentsev, S. and Todd, J.A., 2007. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes 56(10), 2616-2621.

Balasubramanian, S. and Ganesh, R., 2008. Vitamin D deficiency in exclusively breast-fed infants. Indian Journal of Medical Research 127, 252-255.

Basile, L.A., Taylor, S.N., Wagner, C.L., Quinones, L. and Hollis, B.W., 2007. Neonatal vitamin D status at birth at latitude 32 degrees 72': evidence of deficiency. Journal of Perinatology 27(9), 568-571.

Bassir, M., Laborie, S., Lapillonne, A., Claris, O., Chappuis, M.C. and Salle, B.L., 2001. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. Acta Paediatrica 90(5), 577-579.

Belderbos, M.E., Houben, M.L., Wilbrink, B., Lentjes, E., Bloemen, E.M., Kimpen, J.L., Rovers, M. and Bont, L., 2011. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics 127(6), e1513-e1520.

Bin-Abbas, B.S., Jabari, M.A., Issa, S.D., Al-Fares, A.H. and Al-Muhsen, S., 2011. Vitamin D levels in Saudi children with type 1 diabetes. Saudi Medical Journal 32(6), 589-592.

Bishop, N., 2005. Chapter 48. Perinatal vitamin D actions. In: Feldman, D., Pike, J.W. and Glorieux, F., (eds.) Vitamin D. Elsevier Academic Press, San Diego, CA, USA, pp. 803-810.

Bodnar, L.M., Catov, J.M., Roberts, J.M. and Simhan, H.N., 2007. Prepregnancy obesity predicts poor vitamin D status in mothers and their neonates. Journal of Nutrition 137(11), 2437-2442.

Bowyer, L., Catling-Paull, C., Diamond, T., Homer, C., Davis, G. and Craig, M.E., 2009. Vitamin D, PTH and calcium levels in pregnant women and their neonates. Clinical Endocrinology (Oxford) 70(3), 372-377.

Bozzetti, V. and Tagliabue, P. 2009. Metabolic bone disease in preterm newborn: an update on nutritional issues. Italian Journal of Pediatrics 35, 20.

Casey, C.F., Slawson, D.C. and Neal, L.R., 2010. Vitamin D supplementation in infants, children, and adolescents. American Family Physician. 81(6), 745-748.

Challa, A., Ntourntoufi, A., Cholevas, V., Bitsori, M., Galanakis, E. and Andronikou, S., 2005. Breastfeeding and vitamin D status in Greece during the first 6 months of life. European Journal of Pediatrics 164(12), 724-729.

Cifuentes, R.F., Hanson, L.C., Jantzer, J.L., Lussky, R.C. and Adkisson, C.A., 2012. Vitamin D deficiency and insufficiency treatment in preterm Infants. Pediatric Academic Societies Annual Meeting, 4511.127. Available at: http://www.abstracts2view.com/pas/.

Dawodu, A. and Nath, R., 2011. High prevalence of moderately severe vitamin D deficiency in preterm infants. Pediatrics International 53(2), 207-210.

Dawodu, A., Zalla, L., Woo, J.G., Herbers, P.M., Davidson, B., Heubi, J. and Morrow, A.L., 2010. Vitamin D deficiency in breastfeeding mothers and their infants. Pediatric Academics Societies Annual Meeting Abstracts, E-PAS2010:1355.5. Available at: http://www.pas-meeting.org/2010Vancouver/Abstracts/default.asp.

Dawson-Hughes, B., 2004. Racial/ethnic considerations in making recommendations for vitamin D for adult and elderly men and women. American Journal of Clinical Nutrition 80(6), 1763S-1766S.

Diamond, T.H., Eisman, J.A., Mason, R.S., Nowson, C.A., Pasco, J.A., Sambrook, P.A. and Wark, J.D., Working Group of the Australian and New Zealand Bone and Mineral Society, Endocrine Society of Australia and Osteoporosis, 2005. Vitamin D and adult bone health in Australia and New Zealand: a position statement. Medical Journal of Australia 182 (6), 281-285.

Dijkstra, S.H., Van Beek, A., Janssen, J.W., De Vleeschouwer, L.H.M., Huysman, W.A. and Van den Akker, E.L.T., 2007. High prevalence of vitamin D deficiency in newborn infants of high-risk mothers. Archives of Disease in Childhood 92, 750-753.

Dror, D.K. and Allen, L.H., 2010. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. Nutrition Reviews 68(8), 465-477.

Equils, O. and Hewison, M., 2010. A role for vitamin D in placental immunology. Journal of Infectious Diseases 201(12), 1950-1951.

Fewtrell, M.S., Cole, T.J., Bishop, N.J. and Lucas, A., 2000. Neonatal factors predicting childhood height in preterm infants: evidence for a persisting effect of early metabolic bone disease? Journal of Pediatrics 137, 668-673.

First Nations, Inuit and Métis Health Committee, reaffirmed 2012. Vitamin D supplementation: recommendations for Canadian mothers and infants. Available at: http://www.cps.ca/english/statements/ii/fnim07-01.htm.

Fischer, P.R., Thacher, T.D. and Pettifor, J.M., 2008. Vitamin D and rickets beyond America. Archives of Pediatric and Adolescent Medicine 162(12), 1193.

Gallo, S., Jean-Philippe, S., Rodd, C. and Weiler, H.A., 2010. Vitamin D supplementation of Canadian infants: practices of Montreal mothers. Applied Physiology, Nutrition and Metabolism. 35(3), 303-309.

Gallo, S., Vanstone, C.A., Comeau, K., Agellon, S., Rodd, C., Sharma, A. and Weiler, H.A., 2010. Vitamin D dose-response study in Canadian infants: 25-hydroxy-vitamin D status during a 20 week intervention. The FASEB Journal 24 (Meeting Abstract Supplement) 537.11.

Garganta, M.D. and Buchanan, A.O., 2012. Improving the rate of Vitamin D supplementation in infants. Pediatric Academics Societies Annual Meeting Abstracts, 1504.130. Available at: http://www.abstracts2view.com/pas/.

Giapros, V.I., Schiza, V., Challa, A.S., Cholevas, V.K., Theocharis, P.D., Kolios, G., Pantou, C. and Andronikou, S.K., 2012. Vitamin D and parathormone levels of late-preterm formula fed infants during the first year of life. European Journal of Clinical Nutrition 66(2), 224-230.

Ginde, A.A., Liu, M.C. and Camargo Jr., C.A., 2009. Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. Archives of Internal Medicine 169 (6), 626-632.

Goldring, S., Warner, J.O., Shaheen, S.O. and Boyle, R.J., 2011. Early life vitamin D status and lung development. Current Respiratory Medicine Reviews 7(6), 396-403.

Gordon, C.M., Feldman, H.A., Sinclair, L., Williams, A.L., Kleinman, P.K., Perez-Rossello, J. and Cox, J.E., 2008. Prevalence of vitamin D deficiency among healthy infants and toddlers. Archives of Pediatrics and Adolescent Medicine 162(6), 505-512.

Groh-Wargo, S and Sapsford, A., 2009. Enteral nutrition support of the preterm infant in the neonatal intensive care unit. Nutrition in Clinical Practice 24, 363-376.

Gutiérrez, O.M., Farwell, W.R., Kermah, D. and Taylor, E.N., 2012. Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporosis International 22(6), 1745-1753.

Güven, A., Ecevit, A., Sözer, O., Tarcan, A., Tarcan, A. and Ozbek, N., 2012. Correlation between the cord vitamin D levels and regulatory T cells in newborn infants. European Journal of Pediatrics. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22383070.

Hanson, C., Armas, L., Lyden, E. and Anderson-Berry, A., 2011. Vitamin D status and associations in newborn formula-fed infants during initial hospitalization. Journal of the American Dietetic Association 111(12), 1836-1843.

Hatun, S., Ozkan, B., Orbak, Z., Doneray, H., Cizmecioglu, F., Toprak, D. and Calikoglu, A.S., 2005. Vitamin D deficiency in early infancy. Journal of Nutrition 135(2), 279-282.

Hayward, I., Stein, M.T. and Gibson, M.I., 1987. Nutritional rickets in San Diego. Archives of Pediatric and Adolescent Medicine 141(10), 1060-1062.

Henderson, A., 2005. Vitamin D and the breastfed infant. Journal of Obstetric, Gynecological and Neonatal Nursing 34(3), 367-372.

Hirsch, D., Archer, F.E., Joshi-Kale, M., Vetrano, A.M. and Weinberger, B., 2011. Decreased anti-inflammatory responses to vitamin D in neonatal neutrophils. Mediators of Inflammation Epub 2011 Dec 15. Available at: http://www.hindawi.com/journals/mi/2011/598345/.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M., 2012. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. The Journal of Clinical Endocrinology & Metabolism 97(4), 1153-1158.

Holick, M.F., 2006. Resurrection of vitamin D deficiency and rickets. Journal of Clinical Investigation 116 (8), 2062-2072.

Hollis, B.W., Johnson, D., Hulsey, T.C., Ebeling, M. and Wagner, C.L., 2011. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. Journal of Bone and Mineral Research 26(10), 2341-2357.

Holmlund-Suila, E., Andersson, S., Hytinantti, T., Lamberg-Allardt, C., Viljakainen, H. and Mäkitie, O., 2012. High-dose vitamin D intervention in infants – effects on vitamin D status, calcium homeostasis, and bone strength. Journal of Clinical Endocrinology and Metabolism 97(11), 4139-4147.

Huurre, A., Laitinen, K., Hoppu, U. and Isolauri, E., 2006. How practice meets guidelines: evaluation of nutrition counselling in Finnish well-women and well-baby clinics. Acta Paediatrica 95(11), 1353-1359.

Hyppönen E., 2010. Vitamin D and increasing incidence of type 1 diabetes – evidence for an association? Diabetes, Obesity and Metabolism, Review 12(9), 737-743.

Hyppönen, E., Fararouei, M., Sovio, U., Hartikainen, A.L., Pouta, A., Robertson, C., Whittaker, J.C. and Jarvelin, M.R., 2011. High-dose vitamin D supplements are not associated with linear growth in a large Finnish cohort. Journal of Nutrition 141(5), 843-848.

Hyppönen. E., Läärä, E., Reunanen, A., Järvelin, M.R. and Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. The Lancet 358(9292), 1500-1503.

Janner, M., Ballinari, P., Mullis, P.E. and Flück, C.E., 2010. High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. Swiss Medical Weekly September 3;140, w13091.

Kahn, H.S., Morgan, T.M., Case, L.D., Dabelea, D., Mayer-Davis, E.J., Lawrence, J.M., Marcovina, S.M. and Imperatore, G., SEARCH for Diabetes in Youth Study Group, 2009. Association of type 1 diabetes with month of birth among US youth: The SEARCH for Diabetes in Youth Study. Diabetes Care 32(11), 2010-2015.

Kersey, M., Chi, M. and Cutts, D.B., 2011. Anaemia, lead poisoning and vitamin D deficiency in low-income children: do current screening recommendations match the burden of illness? Public Health Nutrition 14, 1424-1428.

Kimball, S., Fuleihan, Gel-H. and Vieth, R., 2008. Vitamin D: a growing perspective. Critical Reviews in Clinical Laboratory Sciences 45(4), 339-414.

Koletzko, B., Baker, S., Cleghorn, G., Neto, U.F., Gopalan, S., Hernell, O., Hock, Q.S., Jirapinyo, P., Lonnerdal, B., Pencharz, P., Pzyrembel, H., Ramirez-Mayans, J., Shamir, R., Turck, D., Yamashiro, Y. and Zong-Yi, D., 2005. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. Journal of Pediatric Gastroenterology and Nutrition 41(5), 584-599.

Koo, W.W., Krug-Wispe, S., Neylan, M., Succop, P., Oestreich, A.E. and Tsang, R.C., 1995. Effect of three levels of vitamin D intake in preterm infants receiving high mineral-containing milk. Journal of Pediatric Gastroenterology and Nutrition 21(2), 182-189.

Kreiter, S.R., Schwartz, R.P., Kirkman Jr., H.N., Charlton, P.A., Calikoglu, A.S. and Davenport, M.L., 2000. Nutritional rickets in African American breast-fed infants. Journal of Pediatrics 137(2), 153-157.

Kumar, G.T., Sachdev, H.S., Chellani, H., Rehman, A.M., Singh, V., Arora, H. and Filteau, S., 2011. Effect of weekly vitamin D supplements on mortality, morbidity, and growth of low birthweight term infants in India up to age 6 months: randomised controlled trial. British Medical Journal 342, d2975.

Lam, H.S., So, K.W. and Ng, P.C., 2007. Osteopenia in neonates: a review. Hong Kong Journal of Paediatrics (new series) 12, 118-124.

Lepage, M.C., Moisan, J. and Gaudet, M., 2004. What do Québec children eat during their first six months? Canadian Journal of Dietetic Practice and Research 65(3), 106-113.

Ling, R.E., Coren, M. and Goldring, S., 2011. Barriers to effective vitamin D supplementation during ante-natal care. British Paediatric Respiratory Society British Society of Paediatric Gastroenterology, Hepatology and Nutrition, Archives of Disease in Childhood 96, A22.

Liu, X., Wang, G., Hong, X., Wang, D., Tsai, H.-J., Zhang, S., Arguelles, L., Kumar, R., Wang, H., Liu, R., Zhou, Y., Pearson, C., Ortiz, K., Schleimer, R., Holt, P.G., Pongracic, J., Price, H.E., Langman, C. and Wang, X., 2011. Gene-vitamin D interactions on food sensitization: a prospective birth cohort study. Allergy 66 (11), 1442-1448.

Madar, A.A., Klepp, K.I. and Meyer, H.E., 2009. Effect of free vitamin D(2) drops on serum 25-hydroxyvitamin D in infants with immigrant origin: a cluster randomized controlled trial. European Journal of Clinical Nutrition 63(4), 478-484.

Maiya, S., Sullivan, I., Allgrove, J., Yates, R., Malone, M., Brain, C., Archer, N., Mok, Q., Daubeney, P., Tulloh, R. and Burch, M., 2008. Hypocalcaemia and vitamin D deficiency: an important, but preventable, cause of life-threatening infant heart failure. Heart 94(5), 581-584.

Manaseki-Holland, S., Maroof, Z., Bruce, J., Mughal, M.Z., Masher, M.I., Bhutta, Z.A., Walraven, G. and Chandramohan, D., 2012. Effect on the incidence of pneumonia of vitamin D supplementation by quarterly bolus dose to infants in Kabul: a randomised controlled superiority trial. The Lancet 379(9824), 1419-1427.

Marjamäki, L., Niinistö, S., Kenward, M.G., Uusitalo, L., Uusitalo, U., Ovaskainen, M.L., Kronberg-Kippilä, C., Simell, O., Veijola, R., Ilonen, J., Knip, M. and Virtanen, S.M., 2010. Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. Diabetologia 8, 1599-1607.

Maxwell, J.D., Ang, L., Brooke, O.G. and Brown, I.R.F., 1981. Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. British Journal of Obstetrics & Gynaecology 88(10), 987-991.

Mehta, S., Hunter, D.J., Mugusi, F.M., Spiegelman, D., Manji, K.P., Giovannucci, E.L., Hertzmark, E., Msamanga, G.I. and Fawzi, W.W., 2009. Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania. Journal of Infectious Diseases 200(7), 1022-1030.

Merewood, A., Grossman, X., Mehta, S.D., Sadacharan, R., Holick, M.F. and Bauchner, H., 2009. Failure to adhere to the AAP's 2003 vitamin D supplementation guidelines for infants. Pediatric Academics Societies Annual Meeting Abstracts, 292. Available at: http://www.abstracts2view.com/pas/.

Monangi, N., Dawodu, A., Slaughter, J., Smith, C. and Akinbi, H., 2012. Vitamin D status in very low birth weight infants and effectiveness of current supplementation strategy. Pediatric Academic Societies Annual Meeting, 2755.1. Available at: http://www.abstracts2view.com/pas/.

Munns, C., Zacharin, M.R., Rodda, C.P., Batch, J.A., Morley, R., Cranswick, N.E., Craig, M.E., Cutfield, W.S., Hofman, P.L., Taylor, B.J., Grover, S.R., Pasco, J.A., Burgner, D. and Cowell, C.T., Paediatric Endocrine Group, Paediatric Bone Australasia, 2006. Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. Medical Journal of Australia 185(5), 268-272.

Munshi, U.K., Kidwai, S.M., Loomis, T. and Rios, A., 2012. Do infants <1500g birth weight achieve the daily recommended intake of vitamin D (VitD) in neonatal intensive care init (NICU)? Pediatric Academic Societies Annual Meeting, 4511.128. Available at: http://www.abstracts2view.com/pas/.

Newberry, R.E., 1982. The infant formula act of 1980. Journal of the Association of Official Analytical Chemists 65(6), 1472-1473.

Onwuneme, C., Carroll, A., McCarthy, R., Kilbane, M., McKenna, M., Murphy, N. and Molloy, E.J., 2012. Towards evidence based medicine for paediatricians. Question 2. What is the ideal dose of vitamin D supplementation for term neonates? Archives of Disease in Childhood 97(4), 387-389.

Pehlivan, Ý., Hatun, Þ., Aydoðan, M., Babaoðlu, K. and Gökalp, A.S., 2003. Maternal vitamin D deficiency and vitamin D supplementation in healthy infants. The Turkish Journal of Pediatrics 45, 315-320.

Perrine, C.G., Sharma, A.J., Jefferds, M.E., Serdula, M.K. and Scanlon, K.S., 2010. Adherence to vitamin D recommendations among US infants. Pediatrics 125(4), 627-632.

Pludowski, P., Socha, P., Karczmarewicz, E., Zagorecka, E., Lukaszkiewicz, J., Stolarczyk, A., Piotrowska-Jastrzebska, J., Kryskiewicz, E., Lorenc, R.S. and Socha, J., 2011. Vitamin D supplementation and status in infants: a prospective cohort observational study. Journal of Pediatric Gastroenterology and Nutrition. 53(1), 93-99.

Ponnapakkam, T., 2010. A treatment trial of vitamin D supplementation in breast-fed infants: universal supplementation is not necessary for rickets prevention in Southern Louisiana. Clinical Pediatrics 49 (11), 1053-1060.

Porcelli, P.J., Helderman, J.B. and Sisk, P.M., 2012. Early postnatal vitamin D and supplementation with serum concentrations in very low birth weight [VLBW] infants. Pediatric Academic Societies Annual Meeting, 4511.129. Available at: http://www.abstracts2view.com/pas/.

Pronzini, F., Bartoli, F., Vanoni, F., Corigliano, T., Ragazzi, M., Balice, P. and Bianchetti, M.G., 2008. Palatability of vitamin D3 preparations modulates adherence to the supplementation in infancy. Clinical Pediatric Endocrinology 17(2), 57-60.

Rodd, C., Jean-Philippe, S., Vanstone, C. and Weiler, H., 2011. Comparison of 2 vitamin D supplementation modalities in newborns: adherence and preference. Applied Physiology, Nutrition, and Metabolism 36(3), 414-441.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. The Journal of Clinical Endocrinology and Metabolism 96(1), 53-58.

Saadi, H.F., Dawodu, A., Afandi, B., Zayed, R., Benedict, S., Nagelkerke, N. and Hollis, B.W., 2009. Effect of combined maternal and infant vitamin D supplementation on vitamin D status of exclusively breastfed infants. Maternal and Child Nutrition 5(1), 25-32.

Salle, B.L., Delvin, E.E., Lapillonne, A., Bishop, N.J. and Glorieux, F.H., 2000. Perinatal metabolism of vitamin D. American Journal of Clinical Nutrition 71(5 Suppl), 1317S-1324S.

Scientific Committee on Food, 2003. Report on the revision of essential requirements of infant and follow-on formulae (adopted on 4 April 2003). Available at: http://europa.eu.int/comm./food/fs/sc/scf/index_en.html.

Shaikh, U. and Alpert, P.T., 2004. Practices of vitamin D recommendation in Las Vegas, Nevada. Journal of Human Lactation 20(1), 56-61.

Shedeed, S.A., 2012. Vitamin D supplementation in infants with chronic congestive heart failure. Pediatric Cardiology 33(5),713-719.

Sherman, E.M. and Svec, R.V., 2009. Barriers to vitamin D supplementation among military physicians. Military Medicine 174(3), 302-307.

Siafarikas, A., Piazena, H., Feister, U., Bulsara, M.K., Meffert, H. and Hesse, V., 2011. Randomised controlled trial analysing supplementation with 250 versus 500 units of vitamin D3, sun exposure and surrounding factors in breastfed infants. Archives of Diseases in Childhood 96, 91-95.

Signorello, L.B., Shi, J., Cai, Q., Zheng, W., Williams, S.M., Long, J., Cohen, S.S., Li, G., Hollis, B.W., Smith, J.R. and Blot, W.J., 2011. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D levels among African Americans. PLoS ONE 6(12), e28623.

Simpson, M., Brady, H., Yin, X., Seifert, J., Barriga, K., Hoffman, M., Bugawan, T., Barón, A.E., Sokol, R.J., Eisenbarth, G., Erlich, H., Rewers, M. and Norris, J.M., 2011. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia 54(11), 2779-2788.

Soliman, A.T., Al Khalaf, F., Alhemaidi, N., Al Ali, M., Al Zyoud, M. and Yakoot, K., 2008. Linear growth in relation to the circulating concentrations of insulin-like growth factor I, parathyroid hormone, and 25-hydroxy vitamin D in children with nutritional rickets before and after treatment: endocrine adaptation to vitamin D deficiency. Metabolism 57(1), 95-102.

Stalgis-Bilinski, K.L., Boyages, J., Salisbury, E.L., Dunstan, C.R., Henderson, S.I. and Talbot, P.L., 2011. Burning daylight: balancing vitamin D requirements with sensible sun exposure. Medical Journal of Australia 194(7), 345-348.

Stearns, G., 1968. Fifty years of experience in nutrition and a look to the future: III. Early studies of vitamin D requirement during growth. American Journal of Public Health and the Nation's Health. 58(11), 2027-2035.

Stephens, B.E., Walden, R.V., Gargus, R.A., Tucker, R., McKinley, L., Mance, M., Nye, J. and Vohr, B.R., 2009. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123(5), 1337-1343.

Sweeney, C., Curtin, K., Murtaugh, M.A., Caan, B.J., Potter, J.D. and Slattery, M.L.I, 2006. Haplotype analysis of common vitamin D receptor variants and colon and rectal cancers. Cancer Epidemiology, Biomarkers and Prevention 15, 744-749.

Taylor, J.A., Geyer, L.J. and Feldman, K.W., 2010. Use of supplemental vitamin D among infants breastfed for prolonged periods. Pediatrics 125(1), 105-111.

Taylor, S.N., Hollis, B.W. and Wagner, C.L., 2009. Vitamin D needs of preterm infants. NeoReviews 10(12), e590-e599.

Taylor, S.N., Wagner, C.L., Anderson, B., Ebeling, M. and Hollis, B.W., 2012b. Vit D status and bone mineralization (BM) in very low birth weight (VLBW) infants at term age (TA). Pediatric Academic Societies Annual Meeting, 4511.132]. Available at: http://www.abstracts2view.com/pas/.

Taylor, S.N., Wagner, C.L., Ebeling, M., Bivens, B. and Hollis, B.W., 2012a. Association of vitamin D status and calcium homeostasis in very low birth weight (VLBW) infants from birth to term age (TA). Peditatric Academic Societies Annual Meeting, 4511.130. Available at: http://www.abstracts2view.com/pas/.

Trussler, K., 2008b. Vitamin D dose-response study to establish dietary requirements in infants: a pilot study. McGill Library and Collections. Available at: http://digitool.library.mcgill.ca/R/-?func=dbin-jump-full&object_id=21913&silo_library=GEN01.

Trussler, K., Rodd, C., Gallo, S., Vanstone, C., Khamessan, A., L'Abbe, M. and Weiler, H., 2008a. Vitamin D dose response study in Canadian infants. Journal of Bone and Mineral Research 23 (supplement 1), S31.

Tylavsky, F.A., Ryder, K.A., Lyytikäinen, A. and Cheng, S., 2005. Vitamin D, parathyroid hormone, and bone mass in adolescents. Journal of Nutrition 135 (11), 2735S-2738S.

Viard, J.P., Souberbielle, J.C., Kirk, O., Reekie, J., Knysz, B., Losso, M., Gatell, J., Pedersen, C., Bogner, J.R., Lundgren, J.D. and Mocroft, A.; EuroSIDA Study Group, 2011. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. AIDS 25(10), 1305-1315.

Vidailhet, M., Mallet, E., Bocquet, A., Bresson, J.L., Briend, A., Chouraqui, J.P., Darmaun, D., Dupont, C., Frelut, M.L., Ghisolfi, J., Girardet, J.P., Goulet, O., Hankard, R., Rieu, D., Simeoni, U. and Turck, D., 2012. Vitamin D: still a topical matter in children and adolescents. A position paper by the Committee on Nutrition of the French Society of Paediatrics.; nutrition de la Archives of Pediatrics 19(3), 316-328.

Viswanathan, S., McNelis, K., Dykstra, C., Amstadt, R., Khasawneh, W., Groh-Wargo, S. and Kumar, D., 2012. Incidence and risk factors for metabolic bone disease in extremely low birth weight infants. Pediatric Academic Societies Annual Meeting, 4524.324. Available at: http://www.abstracts2view.com/pas/.

Wagner, C.L., Hulsey, T.C., Fanning, D., Ebeling, M. and Hollis, B.W., 2006. High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. Breastfeeding Medicine 1(2), 59-70.

Wagner, C.L., Johnson, D., Hulsey, T.C., Ebeling, M., Shary, J., Smith, P.G., Bivens, B. and Hollis, B.W., 2010. Vitamin D supplementation during pregnancy Part 2 NICHD/CTSA randomized clinical trial (RCT): outcomes. Pediatric Academics Societies Annual Meeting Abstracts, E-PAS2010: 1665.6. Available at: http://www.pas-meeting.org/2010Vancouver/Abstracts/default.asp.

Wagner, C.L., Taylor, S.N., Dawodu, A., Johnson, D.D. and Hollis, B.W., 2012. Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients 4(3), 208-230.

Walker, V.P., Zhang, X., Rastegar, I., Liu, P.T., Hollis, B.W., Adams, J.S. and Modlin, R.L., 2011. Cord blood vitamin D status impacts innate immune responses. The Journal of Clinical Endocrinology and Metabolism. 96(6), 1835-1843.

Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., Van Meurs, J.B., Berry, D., Kiel, D.P., Streeten, E.A., Ohlsson, C., Koller, D.L., Peltonen, L., Cooper, J.D., O'Reilly, P.F., Houston, D.K., Glazer, N.L., Vandenput, L., Peacock, M., Shi, J., Rivadeneira, F., McCarthy, M.I., Anneli, P., De Boer, I.H., Mangino, M., Kato, B., Smyth, D.J., Booth, S.L., Jacques, P.F., Burke, G.L., Goodarzi, M., Cheung, C.L., Wolf, M., Rice, K., Goltzman, D., Hidiroglou, N., Ladouceur, M., Wareham, N.J., Hocking, L.J., Hart, D., Arden, N.K., Cooper, C., Malik, S., Fraser, W.D., Hartikainen, A.L., Zhai, G., Macdonald, H.M., Forouhi, N.G., Loos, R.J., Reid, D.M., Hakim, A., Dennison, E., Liu, Y., Power, C., Stevens, H.E., Jaana, L., Vasan, R.S., Soranzo, N., Bojunga, J., Psaty, B.M., Lorentzon, M., Foroud, T., Harris, T.B., Hofman, A., Jansson, J.O., Cauley, J.A., Uitterlinden, A.G., Gibson, Q., Järvelin, M.R., Karasik, D., Siscovick, D.S., Econs, M.J., Kritchevsky, S.B., Florez, J.C., Todd, J.A., Dupuis, J., Hyppönen, E. and Spector, T.D., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376(9736), 180-188.

Will, H., Taylor, S.N. and Wagner, C.L., 2009. Vitamin D requirements during infancy: reading between the lines. Leaven 45(1), 2-6.

Williamson, S. and Greene, S., 2007. Rickets: prevention message is not getting through. British Medical Journal 334(7607), 1288.

Yamshchikov, A.V., Desai, N.S., Blumberg, H.M., Ziegler, T.R. and Tangpricha, V., 2009. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocrine Practice 15(5), 438-449.

Zamora, S.A., Rizzoli, R., Belli, D.C., Slosman, D.O. and Bonjour, J.P., 1999. Vitamin D supplementation during infancy is associated with higher bone mineral mass in prepubertal girls. Journal of Clinical Endocrinology and Metabolism 84(12), 4541-4544.

Ziegler, E.E., Hollis, B.W., Nelson, S.E. and Jeter, J.M., 2006. Vitamin D deficiency in breastfed infants in Iowa. Pediatrics 118(2), 603-610.

Zipitis, C.S. and Akobeng, A.K., 2008. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Archives of Disease in Childhood 93(6), 512-517.

Key facts

- Vitamin D deficiency rickets is a disease observed most commonly in infants when infants and their mothers have limited sun exposure and have limited or no vitamin D intake.
- Vitamin D deficiency in infants is not only associated with the bone deformity of rickets, but also with hypocalcemic seizures and cardiomyopathy
- Infant vitamin D intake of at least 400 IU/day is associated with avoidance of vitamin D deficiency rickets.
- Small, pilot studies of maternal vitamin D intake of 2,000 to 6,400 IU/day have shown that breast milk can adequately provide vitamin D supplementation to the infant.
- Ongoing studies will establish the maternal vitamin D intake that safely and effectively supports infant vitamin D health with breastfeeding.

Summary points

- During lactation, breast milk calcium is provided predominantly from maternal bone resorption and this resorption is controlled by increased parathyroid related protein and decreased estradiol.
- The vitamin D activity passed during lactation from mother to infant is mostly in the forms of vitamin D and the metabolite 25-hydroxyvitamin D.
- For mothers receiving 200-400 IU/day vitamin D, breast milk vitamin D activity concentration is 20-70 IU/l which is far less than United States infant formula concentration of at least 400 IU/l vitamin D.
- The American Academy of Pediatrics recommends 400 IU/day vitamin D supplementation for breastfeeding infants.
- The American Academy of Pediatrics defines vitamin D deficiency as 25-hydroxyvitamin D <50 nmol/l.
- Risk factors for vitamin D deficiency for mother and infant during lactation include dark pigmentation, limited sun exposure, sunscreen use, high latitude, winter season, limited maternal intake during pregnancy and lactation, and absence of infant vitamin D supplementation.
- Vitamin D deficiency for breastfeeding infants is a global problem even in sun-rich environments.
- Case-control studies of infant or child vitamin D status and acute lower respiratory infections demonstrate an association between acute lower respiratory infection and 25-hydroxyvitamin D status <22.5 nmol/l.
- Maternal vitamin D intake and status may be adequately elevated to supply vitamin D to the breastfed infant through breast milk.

32. Lactation and vitamin D

S.N. Taylor, C.L. Wagner and B.W. Hollis
The Darby Children's Research Institute and the Department of Pediatrics, Medical University of South Carolina, 165 Ashley Avenue, MSC 917, Charleston, SC 29425, USA; taylorse@musc.edu

Abstract

The resurgence in vitamin D-associated rickets and world-wide description of maternal and infant vitamin D deficiency highlights the need for global maternal and infant vitamin D recommendations and action to ensure vitamin D health during lactation. Direct infant vitamin D supplementation of at least 400 IU/day vitamin D will protect the majority of infants from vitamin D deficiency rickets by maintaining 25-hydroxyvitamin D status above 50 nmol/l regardless of race, season, latitude, or maternal vitamin D status. Additionally, the physiology of breast milk production comprises the mechanism required for the mother to supply vitamin D activity to her infant and, with adequate maternal vitamin D intake, a mother is able to supply her infant with the vitamin D required to match the status achieved with direct infant supplementation. As current multi-center, randomized, controlled trials are underway to identify the maternal vitamin D supplementation associated with safe, effective supplementation of the breastfed infant, studies to more accurately define infant vitamin D health also are occurring. Though the vitamin D status and dose to safely avoid vitamin D deficiency rickets are known, the vitamin D status and/or dose (infant and/or maternal) that optimizes infant bone health or immune function for both short- and long-term outcomes has yet to be identified. Current case series and cohort studies demonstrate that infant vitamin D health may be important in avoiding acute lower respiratory infection and diabetes mellitus type 1.

Keywords: breastfeeding, infant, mother, rickets

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_32, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
AAP	American Academy of Pediatrics
ALRI	Acute lower respiratory infection
IOM	US Institute of Medicine
UVB	Ultraviolet B

32.1 Introduction

Though vitamin D's role in preventing rickets has been known for centuries, awareness of contemporary vitamin D science is in its infancy. In the current state, epidemiologic studies and case series raise grave concern that a large proportion of the world lives in a vitamin D-deficient state with associated pathology. Randomized, controlled trials are being performed to evaluate whether the associations described in epidemiologic studies and case series have a biological cause and effect relationship. In investigation of the role of vitamin D in maternal and infant health, direct supplementation to the breastfed infant to avoid vitamin D deficiency and rickets is supported by a number of randomized clinical trials. However, trials addressing functional outcomes such as bone mineralization or immune function are currently smaller in both size and quantity and, therefore, possess less strength of evidence. For investigation of maternal vitamin D supplementation to support the mother and the breastfeeding infant, randomized, controlled trials also are underway. The ecological paradox that the ideal infant nutrition, mother's milk, lacks a nutrient whose absence is associated with severe bone disease will soon be confirmed or invalidated. An overview of this science of vitamin D in lactation is not only informative, but also provides an example of how scientific method, health policy, and cultural evolution have interacted to both impede and advance knowledge. Review of vitamin D and calcium physiology in lactation, epidemiologic studies of global vitamin D status, and trials investigating the outcomes associated with vitamin D supplementation provide the background to understand the current expert-consensus vitamin D guidelines and potential for far greater knowledge in the near future.

32.2 Vitamin D and calcium physiology during lactation

During pregnancy, the calcium needs of the fetus are supplied by a large increase in maternal intestinal calcium absorption and, to a smaller degree, maternal bone mineral resorption especially in the last months of pregnancy (Kalkwarf and Specker, 2002; Kovacs, 2011). Dissimilarly, during lactation, mother's intestinal calcium absorption returns to the pre-pregnancy state (Kalkwarf and Specker, 2002; Kovacs, 2011). The calcium needs of the breastfeeding infant are met predominantly by elevated maternal resorption of calcium and phosphorus from bone and, to a smaller extent, renal reabsorption of calcium (Kent *et al.*, 1990; Specker *et al.*, 1994). The primary mechanisms of control of the elevated bone resorption are mammary gland production of parathyroid related protein and the lactation-associated decrease in estradiol (Ardeshirpour *et al.*, 2010; Kovacs,

2011; Sowers *et al.*, 1996). These two mechanisms appear to work synergistically (Ardeshirpour *et al.*, 2010). Through two to six months of lactation, these and likely other mechanisms are responsible for a loss of 5-10% of maternal bone density (Kalkwarf and Specker, 2002; Kovacs, 2011). Following weaning, bone density is rapidly regained to pre-pregnancy status and women who lactate have no long-term ramifications from this bone loss and, unexpectedly, lactation may have a protective effect on post-menopausal bone health (Kalkwarf and Specker, 2002; Polatti *et al.*, 1999; Sowers, 1996). Of note, increasing maternal calcium intake during lactation has no effect on the rate of bone resorption, and increased calcium intake post-weaning does not affect the rate of bone mineral recovery (Fairweather-Tait *et al.*, 1995; Kalkwarf and Specker, 2002; Kovacs, 2008).

Just as calcium intake does not affect the concentration of calcium supply from mother to the infant, vitamin D intake does not affect breast milk calcium levels This absence of a relationship between maternal vitamin D status and milk calcium concentration was demonstrated in a study of lactating mothers receiving 2,000 or 4,000 IU/day vitamin D from one to four months postpartum. No association was seen between maternal vitamin D dose, maternal 25(OH)D status, and milk calcium concentration (Basile *et al.*, 2006). Prior to this study, concern was raised that vitamin D supplementation of the mother would decrease bone resorption and, thereby, decrease milk calcium concentration.

Although maternal vitamin D status does not demonstrate a significant effect on milk calcium concentration, her vitamin D status has a great role in her milk vitamin D supply. The vitamin D activity or antirachitic activity that a mother passes to her child through breast milk is a combination of the parent compound, vitamin D, and its metabolites. The compound in highest concentration in human milk is vitamin D. Thirty percent of maternal vitamin D and 1% of maternal 25(OH)D are secreted in the milk. Very minimal amounts of other metabolites including the active metabolite, 1,25-dihydroxyvitamin D are present in the milk. Mother's cutaneously-produced vitamin D3 and its metabolite, 25(OH)D3, as well as any oral intake of vitamin D3 or vitamin D2 and their 25(OH)D forms are present in milk (Greer *et al.*, 1984a; Hollis, 1983; Hollis *et al.*, 1981; Reeve *et al.*, 1982; Weisman *et al.*, 1982).

Lactation represents a specific time in women's health when daily intake or production of vitamin D is required. Since the parent compound comprises the majority of the antirachitic activity and this compound has a relatively short half-life, regular dosing is required to guarantee consistency in the vitamin D activity supply to the infant. In other circumstances, weekly or monthly vitamin D dosing may be efficacious, but not during lactation. In fact, the balance of vitamin D and 25(OH)D in milk is well-suited to the needs of the infant. The 1% of 25(OH)D supports a steady supply of antirachitic activity, while the larger supply of vitamin D allows instantaneous passage of maternal vitamin D intake or cutaneous production (Greer *et al.*, 1984a).

Of note, with maternal vitamin D supplementation of 200-400 IU/day vitamin D, mother's milk contains 33-68 IU/l antirachitic activity (Hollis, 1983; Hollis *et al.*, 1981; Wagner *et al.*, 2006). This supply of vitamin D is well below the AAP recommendations of 400 IU/day for infants

(Wagner and Greer, 2008). However, several studies clearly demonstrate the potential for vitamin D distribution from mother to infant. In 1984, Greer *et al.* (1984b) published a report of a mother with hypoparathyroidism who received 100,000 IU/day vitamin D2 treatment. As reported in other studies, even with this higher intake, mother's milk contained 30% of her circulating vitamin D concentration and 1% of her circulating 25(OH)D concentration. The antirachitic activity in the milk was 7,660 IU/l.

Cutaneous production of vitamin D from maternal UVB (sunlight) exposure also has been shown to increase the vitamin D content of milk. A study that involved exposing lactating white women to a dose of total body UVB equal to 30 minutes of sunshine at midday on a clear summer day at temperate latitudes demonstrated an increase in vitamin D content of milk with a peak at 48 hours following the exposure and a return to baseline vitamin D activity at seven days. Of note, milk 25(OH)D concentration did not vary, but instead all variation was due to the vitamin D compound concentration in the milk. Additionally, infant 25(OH)D status rose from 34.8 to 51.3 nmol/l (Greer *et al.*, 1984a).

32.3 Vitamin D recommendations for infants

Though these studies describe both oral supplementation and sun-exposure to increase mother's vitamin D supply to her nursing infant, globally, current maternal vitamin D status does not provide adequate antirachitic activity in milk for all breastfeeding infants to avoid rickets. Rickets is a debilitating bone disease including bowing of legs and curvature of the spine, widening of metaphyses and costochondral joints, and enlargement of the skull. Additionally, severe vitamin D deficiency may present with generalized muscle weakness, cardiomyopathy or hypocalcemic seizures (Balasubramanian *et al.*, 2006; Dunn, 1998; Maiya *et al.*, 2008; Wagner and Greer, 2008). In the past decade, numerous case reports and series from around the world describe a resurgence in vitamin D deficient-rickets (Kreiter *et al.*, 2000; Tomashek *et al.*, 2001; Welch *et al.*, 2000). The number of these reports have risen at the same time as awareness of vitamin D function has grown and as normative values are revised to reflect optimal vitamin D function instead of population norms.

The resurgence in rickets and vitamin D-deficiency associated pathology is partly due to misunderstandings of vitamin D propagated through the 20th century. One of the largest misconceptions was description of vitamin D intake as the cause of supravalvular aortic stenosis (Friedman, 1967; Taussig, 1966). Later discovery demonstrated that this cardiac lesion was due to a genetic anomaly, Williams Syndrome, and not vitamin D intake (Morris and Mervis, 2000). Another precipitating factor in the resurgence of rickets was the decrease in sun exposure in contemporary lifestyle. Both progression to in-door living and use of sunscreen have propagated vitamin D deficiency. Additionally, lack of consistent science describing the vitamin D intake required for breastfeeding infants to avoid rickets has hampered standardization of vitamin D intake recommendations.

Risk factors for rickets are listed in Table 32.1 and include factors associated with decreased UVB exposure and factors associated with decreased vitamin D intake (Dawodu and Tsang, 2007; Wagner and Greer, 2008). A large epidemiologic study in China attempted to identify the vitamin D intake required to avoid rickets. They found no incidence of rickets at 6 months of age in infants receiving as low as 100 IU/day vitamin D supplementation (Specker *et al.*, 1992). Therefore, in 1997, the IOM recommended 200 IU/day as the adequate intake to prevent vitamin D deficiency in normal infants, children, and adolescents (IOM, Food and Nutrition Board, 1997), and the AAP reaffirmed this recommendation by stating that a minimum of 200 IU/day vitamin D should be begun in the first 2 postnatal months (Gartner and Greer, 2003).

Following the recommendation for 200 IU/day infant vitamin D supplementation, United States formula companies continued vitamin D supplementation to provide >400 IU when an infant receives at least one liter of formula. Most formulas in Asia and Europe also contain at least this concentration. The discrepancy between national recommendations in vitamin D supplementation and the vitamin D supplied by infant formula led to a perceptible increase in vitamin D deficiency-rickets specific to the breastfeeding population.

In response to the increased prevalence of rickets, in 2008, the AAP revised its guidelines to recommend 400 IU/day vitamin D to begin in the first postnatal days. This dose was chosen because it is adequate to avoid vitamin D deficiency defined as a 25-hydroxyvitamin D level <50 nmol/l (20 ng/ml). A 25(OH)D level of 50 nmol/l was chosen to define vitamin D deficiency based on the fact that cases of vitamin D-deficiency rickets are reported with 25(OH)D status below this level (Wagner and Greer, 2008). However, the infant vitamin D status that promotes optimal bone mineralization has yet to be defined. Evaluations of an association between vitamin D intake and/or status and bone mineralization are limited by differences in 25(OH)D assay and bone health measurement and demonstrate inconsistent results (Greer and Marshall, 1989; Greer *et al.*, 1982; Kim *et al.*, 2010; Park *et al.*, 1998; Savino *et al.*, 2011).

Table 32.1. Risk factors associated with vitamin D deficiency and/or rickets worldwide.

Limited sunlight exposure	Dark pigmentation
	Limited skin exposure due to body covering practices
	Sunscreen use
	Indoor lifestyle
	High latitude
	Winter season
Limited vitamin D intake	Limited maternal intake during pregnancy
	Limited maternal intake during lactation
	No infant intake

Following the 2008 AAP policy statement, in 2010, the IOM endorsed the AAP guidelines and defined 400 IU/day as adequate intake for infants 0-12 months of age (Ross, 2011). The recommended dietary allowance for children at or above 1 year of age is 600 IU/day. For mothers, the IOM vitamin D recommended dietary allowance for women 14-50 years old is 600 IU/day. The upper level intake for mothers is defined as 4,000 IU/day (Ross, 2011).

32.4 Prevalence of vitamin D deficiency in breastfeeding infants

Recurrence of rickets in the breastfeeding population has led to evaluation of vitamin D status in breastfeeding infants and their mothers. As mentioned previously, rickets has been diagnosed in infants with vitamin D status below 50 nmol/l (Wagner and Greer, 2008). However, many studies of infant vitamin D status define vitamin D deficiency at far lower levels of 25(OH)D.

The risk for vitamin D deficiency rickets likely starts with vitamin D deficiency in the fetal environment and categorically starts with vitamin D deficiency at birth. Consistently, birth status correlates with later vitamin D status in the un-supplemented or under-supplemented infant (Bhalala *et al.*, 2007; Challa *et al.*, 2005; Sachan *et al.*, 2005). Vitamin D status at birth is consistently low with description of mean cord blood levels of 21 nmol/l (Sachan *et al.*, 2005) and 48.4 nmol/l (Bhalala *et al.*, 2007) in India, a range of 10-80 nmol/l with 63.7% below 30 nmol/l in France (Zeghoud *et al.*, 1997), 44.3 nmol/l in Korea (Kim *et al.*, 2010), and, in the United States, mean levels of 26.3 nmol/l in African American infants and 49 nmol/l in Caucasian infants, respectively (Basile *et al.*, 2007). Additionally, Basile *et al.* showed a significant seasonal variation in cord blood 25(OH)D for Caucasian but not African American infants. Compared to birth in April through October, birth in November through March was associated with 28.3 nmol/l lower vitamin D status for Caucasian infants and only 7.5 nmol/l lower status for African American infants (Basile *et al.*, 2007). Therefore, dark pigmentation and winter season are important risk factors for vitamin D deficiency not only during lactation but also prior to lactation in the fetal period.

Studies examining vitamin D status throughout lactation show persistence if not worsening of vitamin D deficiency. The Bhalala *et al.* (2007) study, defining vitamin D deficiency as a 25(OH)D level <62.5 nmol/l, found a vitamin D deficiency prevalence of 62% at birth and 80% at 3 months in breastfeeding infants. In Greece, Challa *et al.* (2005) demonstrated a significant seasonal difference in vitamin D status at 1 week of age with means of 16.8 and 25.3 nmol/l in winter- and summer-born infants, respectively. Of note, during 6-month follow-up, winter-born infants had a mean increase in 25(OH)D to 48.5 nmol/l and summer-born infants had no change. Other studies have shown no seasonal variation in infant vitamin D status (Jain *et al.*, 2011; Saadi *et al.*, 2009). Further examples of vitamin D deficiency in breastfeeding infants are presented in Table 32.2.

In the United States, a breastfeeding study in Iowa found an overall prevalence of 10% vitamin D deficiency (defined as 25(OH)D <27.5 nmol/l). In the winter, 78% of unsupplemented infants

Table 32.2. Studies presenting vitamin D status of lactating mothers and their infants.

Study	Age of infant	Infant 25(OH)D status (nmol/l)	Maternal status (nmol/l)
Dawodu *et al.*, 2003 (United Arab Emirates)	Median 6 weeks (4-16 weeks)	Median 11.5	Median 21.5
Madar *et al.*, 2009 (non-Western immigrants in Norway)	6 weeks	Mean 41.7	Mean 25.8
Jain *et al.*, 2011 (India)	Mean 13.6 weeks	Median 25.3	Median 24.5
Seth *et al.*, 2009 (India)	2-24 weeks	Mean 29	Mean 27.3
Agarwal *et al.*, 2010 (India)	10 weeks	Mean 28.9	Mean 24.6
	6 months	Mean 42.4	Not measured

were vitamin D deficient, while in the summer only one infant with dark pigmentation exhibited deficiency (Ziegler *et al.*, 2006). Additionally, the NHANES survey 2001-2004 demonstrated a 9% rate of vitamin D deficiency (25(OH)D <37.5 nmol/l) and a 61% rate of vitamin D insufficiency (25(OH)D 37.5-72.5 nmol/l) in a national sample of children age 1-21 (Kumar *et al.*, 2009). Though vitamin D status was not evaluated with breastfeeding status, this NHANES survey reveals that vitamin D deficiency is not a problem isolated to countries without standard vitamin D fortification of milk products.

32.5 Effects of vitamin D deficiency in breastfeeding infants

Evaluation of the relationship of vitamin D status with markers of calcium and phosphorus homeostasis, such as calcium, phosphorus, parathyroid hormone, and alkaline phosphatase status, have shown inconsistent results for breastfeeding infants (Challa *et al.*, 2005; Dawodu *et al.*, 2003; Jain *et al.*, 2011; Kim *et al.*, 2010; Madar *et al.*, 2009; Seth *et al.*, 2009; Ziegler *et al.*, 2006). Therefore, at this time, the biochemical outcomes associated with vitamin D deficiency and its effects on bone mineralization and calcium homeostasis are not well-described until a child reaches the severe stages as demonstrated by rickets, hypocalcemic seizures, or heart failure (Wagner and Greer, 2008).

In assessment of bone health and vitamin D status of infants, observational studies have demonstrated a 16% prevalence of radiographic rickets with vitamin D deficiency (Agarwal *et al.*, 2010; Jain *et al.*, 2011). A study using quantitative ultrasound to assess bone status demonstrated lower markers of bone health in breastfeeding infants who did not receive vitamin D supplementation. However, the parameters also demonstrated significant differences based on infant length and/or weight (Savino *et al.*, 2011). Therefore, further prospective evaluation with contemporary bone assessment technology is required. As mentioned previously, randomized trials have not consistently shown improvement in bone health with vitamin D supplementation

(Greer and Marshall, 1989; Greer *et al.*, 1982; Kim *et al.*, 2010; Park *et al.*, 1998). In investigation of the role of infant vitamin D intake in long-term bone health, a study of bone mineralization in prepubertal girls found higher femoral but not spinal bone mineralization with receipt of vitamin D supplementation in the first postnatal year (Zamora *et al.*, 1999).

Assessment of the role of vitamin D in other infant health outcomes has raised concern that vitamin D deficiency is associated with respiratory, infectious outcomes, and autoimmune outcomes. One large cohort study evaluated the long-term effect of vitamin D intake in the first postnatal year and found an 80% decrease in the risk for diabetes type I for infants who received at least 2,000 IU/day vitamin D though the first year (Hypponen *et al.*, 2001). This study has led to ongoing investigation of the physiological cause for this potential relationship.

In case-control studies examining whether an association between infant or children vitamin D status and ALRI exists, the likelihood of finding differences between cases and controls appears to be related to the underlying vitamin D status of the study population. Very interestingly, three studies have demonstrated significant differences between cases and controls with mean 25(OH)D status of 22.8-29.1 nmol/l for cases versus 38.4-40.8 nmol/l for controls (Karatekin *et al.*, 2009; Roth *et al.*, 2010; Wayse *et al.*, 2004). In studies performed in populations with higher vitamin D status, no statistical or clinical difference is appreciated in vitamin D status between cases and controls (77.2-81 vs. 77-83 nmol/l, respectively) (McNally *et al.*, 2009; Roth *et al.*, 2009). Of note, in the studies demonstrating a significant difference in 25(OH)D status between ALRI cases and matched controls, Karatekin *et al.* evaluated neonates and found mothers also demonstrated significantly different vitamin D status (mean 33.5 vs. 57 nmol/l, respectively) (Karatekin *et al.*, 2009). Also, in the Wayse *et al.* study, 25(OH)D status >22.5 nmol/l and exclusive breastfeeding in the first 4 postnatal months were both individually associated with lower odds for severe ALRI (Wayse *et al.*, 2004). Therefore, the potential benefit for vitamin D status to decrease ALRI risk appears to occur by raising vitamin D status from the 22.8-29.1 nmol/l range to at least the 38.4-40.8 nmol/l range.

32.6 Direct vitamin D supplementation to the breastfeeding infant

Based on these studies of infant health outcomes, vitamin D status with 25(OH)D >50 nmol/l appears to support avoidance of the severe negative effects of vitamin D deficiency. Therefore, establishing the vitamin D dose that promotes avoidance of vitamin D deficiency for all populations is critical. The dose of 400 IU/day, currently recommended for infants in the United States, appears to achieve avoidance of vitamin D deficiency. However, defining vitamin D sufficiency and, therefore, the vitamin D status that optimizes outcomes such as bone mineralization is not known. As studies are performed to determine vitamin D sufficiency for breastfeeding infants, recommendations for optimal vitamin D status and vitamin D intake may be revised, but currently 400 IU/day is the goal (Wagner and Greer, 2008).

Contemporary studies of the effect of 400 IU/day supplementation on the vitamin D status of breastfeeding infants are few and are shown in Table 32.3. In the Wagner *et al.* (2010) study, infants entered the study at 1 month of age and mean 25(OH)D at that time was 40 nmol/l. By the next evaluation at 4 months of age, 25(OH)D status had risen to a mean of 109 nmol/l and this increase in status persisted through the 6-month study time period.

Three further studies have evaluated other concentrations of oral vitamin D dosing or UVB exposure. Ho *et al.* (1985) randomized infants one to eight months of age who were exclusively breastfeeding without vitamin D supplementation to receive usual sunlight exposure or to receive a regimented 2 hours of sunshine per day. Over the 2-month study period, 25(OH)D status did not significantly vary in controls but increased in the 2-hour sunlight exposure group. Additionally, the final 25(OH)D status correlated with UVB exposure with estimation that daily 24 minutes of only face exposure will maintain a serum 25(OH)D level of 27.5 nmol/l. The authors did not describe the ultraviolet light exposure necessary to maintain a level of 50 nmol/l (Ho *et al.*, 1985).

In Korea, a randomized, controlled study investigated the effect of 200 IU/day vitamin D supplementation to exclusively breastfed infants. The infants started with a mean 25(OH)D status of 44.3 nmol/l at birth. Over a 12-month study period, unsupplemented and supplemented breastfed infants' 25(OH)D status improved to 83 and 107 nmol/l, respectively. A third group of formula-fed infants achieved mean serum 25(OH)D status of 122 nmol/l. This study also showed significantly higher bone mineral density in formula-fed infants compared to both the unsupplemented and 200 IU/day supplemented breast-fed infants reiterating the need for further evaluation of the relationship of vitamin D status and bone mineralization beyond the diagnosis of rickets (Kim *et al.*, 2010).

Despite this evidence of improved vitamin D status with vitamin D supplementation and the resurgence of rickets in the breastfed-infant population, many pediatric care providers and families are resistant to supplement vitamin D to the breastfeeding infant. Studies examining the rates of pediatric provider recommendation for vitamin D supplementation of breastfed infants have demonstrated only 36.4% and 44.6% recommendation and, in another study, 52.3% of providers stated no recommendation (Davenport *et al.*, 2004; Shaikh and Alpert, 2004; Taylor *et*

Table 32.3. Studies measuring the effect of 400 IU/day breastfeeding infant vitamin D intake.

Study	Serum 25(OH)D with no vitamin D supplement (nmol/l)	Serum 25(OH)D with 400 IU/day vitamin D (nmol/l)	Length of supplementation
Greer *et al.*, 1982	32.3	81.8	Birth to 6 months
Greer and Marshall, 1989	58.8	92.5	Birth to 6 months
Wagner *et al.*, 2010	Not studied	106.3	1 to 7 months

al., 2010). In fact, in North Carolina, where 25.8% of surveyed AAP members reported seeing at least 1 case of vitamin D deficient rickets within the previous 3 years, only 44.6% recommended vitamin D supplementation for all breastfed infants and 38.6% recommended for some breastfed infants, but 16.5% never recommended vitamin D (Davenport *et al.*, 2004). Both pediatric provider and parental reasons for avoiding vitamin D supplementation of the breastfed infant include belief that vitamins are unnecessary because breast milk has all needed nutrition, concern for cost, concern for hypervitaminosis D, and concern that recommending supplementation would decrease the likelihood for successful breastfeeding (Davenport *et al.*, 2004; Taylor *et al.*, 2010).

The need for direct vitamin D supplementation of the breastfed infant does not make ecological sense. If breast milk is deficient in this key nutrient, how have breastfed infants survived for thousands of years? The answer may be that maternal vitamin D deficiency is a modern problem. In the past, populations lived in sun-rich environments or had a regular dietary source high in vitamin D such as fatty fish. Therefore, is it possible that human milk is designed to be sufficient in vitamin D and to provide complete nutrition to the breastfed infant?

32.7 Vitamin D supplementation of mother to support the breastfed infant

As mentioned previously in this chapter, study of maternal vitamin D supplementation to adequately support infant vitamin D health has been limited by unfounded concerns regarding vitamin D safety and, specifically, the IOM 1997 definition of 2,000 IU/day as the upper tolerable intake limit (IOM, Food and Nutrition Board, 1997; Taussig, 1966). For example, Alahoula *et al.* (1986) in Finland compared maternal intake of 2,000 IU/day versus infant intake of 400 IU/day and demonstrated equivalent vitamin D status of infants. Maternal vitamin D status with 2,000 IU/day increased from a mean of 27.5 nmol/l to near 100 nmol/l and infant status improved from a mean of 21.3 nmol/l to near 75 nmol/l. This improvement in infant status matched that observed in the group of infants receiving 400 IU/day directly. Although these results demonstrated that breast milk could adequately support the vitamin D health of the infant, they essentially were ignored for 20 years because as the authors commented, 'As such a dose is far higher than the daily dietary allowance recommended for lactating mothers its safety over prolonged periods is not known and should be examined' (Ala-Houhala *et al.*, 1986).

The current IOM guidelines define 4,000 IU/day as the upper level intake and acknowledge that the specific needs for pregnant and lactating women have yet to be identified (Ross, 2011). Therefore, with progression towards recognition that higher doses of vitamin D are safe and realization that, with these doses, the opportunity to supplement mother to avoid supplementation of the infant exists, contemporary research to identify the vitamin D supplementation of mother that promotes vitamin D health for the infant is underway. Again, progress in this field has been limited by toxicity concerns. In fact, the United States Food and Drug Administration approval for an Investigational New Drug was required for studies of vitamin D intake of 4,000 IU/day or greater during lactation. This approval was the first ever required for the study of a vitamin.

Building from the work by Ala-Houla *et al.* (1986), Hollis and Wagner (2004), published their study evaluating the ability of 2,000 IU/day and 4,000 IU/day vitamin D to support both mother and breastfeeding infant 25(OH)D status by providing all subjects with 400 IU/day vitamin D3 and then additional 1,600 IU/day and 3,600 IU/day given as vitamin D2, respectively. This methodology allowed investigation of the amount of 25(OH)D increased by the extra oral supplementation above the standard 400 IU/day vitamin D3 and the endogenous production of vitamin D3 by both mother and infant. Mother/infant dyads were enrolled at 1 month postpartum if exclusively breastfeeding and were followed for 3 months. The results achieved in this study are presented in Table 32.4. Of note, the infant's vitamin D status in both groups achieved means above 50 nmol/l which may point to 2,000 IU/day as sufficient maternal dose to support the breastfeeding infant. However, only 22 and 39% of infant 25(OH)D status was 25(OH)D2 which means infants likely received vitamin D from other sources such as feed supplementation with infant formula.

This study demonstrated important factors. Firstly, it demonstrated the passage of vitamin D and its antirachitic metabolites from mother, through the milk, to the infant in the vitamin D2 form. Secondly, for both mother and infant, serum calcium concentrations remained in normal range and no hypercalciuria was observed. However, abnormal calcium chemistries were not expected as all subjects' vitamin D status remained below potentially toxic levels (Hollis and Wagner, 2004).

This study was followed by another published in 2006 comparing high- vs. low-dose vitamin D supplementation of mother. In this study, mother/infant dyads were randomized to receive

Table 32.4. Vitamin D status during lactation with mothers receiving 2,000 or 4,000 IU/day (Hollis and Wagner, 2004).

Variable	Vitamin D status	Study time point	1,600 IU/day vitamin D2 and 400 IU/day vitamin D3	3,600 IU/day vitamin D2 and 400 IU/day vitamin D3
Mother	Total 25(OH)D[1]	Baseline	69	82.3
		3 months	90.3	111.3
	25(OH)D2	Baseline	1	4.5
		3 months	43.5	62.5
Infant	Total 25(OH)D	Baseline	19.8	33.5
		3 months	69.5	77
	25(OH)D2	Baseline	<1.3	2
		3 months	15	30
Milk	Antirachitic activity[2]	Baseline	35.5	40.4
	Antirachitic activity	3 months	69.7	134

[1] 25(OH)D given as nmol/l.

[2] Antirachitic activity given as IU/l.

maternal intake of 6,400 IU/day and no direct infant supplementation versus maternal intake of 400 IU/day and infant intake of 300 IU/day. Enrollment occurred at one month postpartum and subjects were followed for 6 months. The primary goal of the study was to determine whether vitamin D supplementation of 6,400 IU/day would result in circulating values of 25(OH)D exceeding 225 nmol/l which, at that time, was chosen as the upper limit of normal. The maternal vitamin D status achieved in the 6,400 IU/day group was an increase from a mean of 85 to 147 nmol/l. This increase was significantly different from the vitamin D status observed for the mothers receiving 400 IU/day for 6 months who showed a change in mean status from 80.8 to 96 nmol/l. For the milk antirachitic activity of the mothers receiving 6,400 IU/day, the activity increased from 82 IU/l at baseline to 873 IU/l after 6 months. The antirachitic activity of the control mothers' milk did not significantly vary over the study period (45.6-78.6 IU/l) and had a nadir of 45.6 IU/l at 5-months postpartum. In evaluation of infant vitamin D status, the infants whose mother's received 6,400 IU/day achieved very similar 25(OH)D concentrations (increase from baseline of 35 to 115 nmol/l) to the infants who received 300 IU/day direct supplementation with mothers receiving 400 IU/day (increase from baseline of 32.5 to 107 nmol/l). In evaluation of safety, no mothers exceeded 225 nmol/l, and maternal and infant serum calcium and phosphorus levels and urinary calcium to creatinine were all within normal range (Wagner *et al.*, 2006).

Two further studies have been performed in the United Arab Emirates (UAE). Despite the sun-rich environment of the UAE, many mothers exhibit vitamin D deficiency and infantile vitamin D rickets is common due to limitations in maternal sun exposure due to conservative clothing practices and to the lack of vitamin D fortification of food products (Saadi *et al.*, 2007). In the first study, Saadi *et al.* (2007) examined the effect of 2,000 IU/day or 60,000 IU/month vitamin D intake on the vitamin D status of nulliparous and lactating women. The investigators used vitamin D2 supplementation as that was the only high-dose supplementation available in the UAE at the time. Additionally, the oral intake of 2,000 IU/day was chosen to not exceed the IOM upper tolerable intake limit.

Overall, at baseline, mean 25(OH)D for lactating women was 25.2 nmol/l. All women except one had 25(OH)D <50 nmol/l and one-third had levels <20 nmol/l. From baseline to the 3 month evaluation, mean 25(OH)D increased by 12.4 nmol/l in the daily intake group and by 14.8 nmol/l in the monthly intake group. For both groups, serum calcium concentrations increased, but did not exceed the normal range. One subject in the monthly intake group exhibited elevation of urinary calcium to creatinine ratio (Saadi *et al.*, 2007).

In a follow-up, Saadi *et al.* (2009) published an evaluation of healthy breastfeeding mothers and their infants with the same maternal vitamin D dosing regimens. Infants in both groups received 400 IU/day. Over a 3 month study period, infant 25(OH)D status increased from 13.9 to 49.6 nmol/l when mothers received 2,000 IU/day and from 13.7 to 44.6 nmol/l when mothers received 60,000 IU/month. Milk antirachitic activity increased from undetectable (<20 IU/l) to 50.9 IU/l (Saadi *et al.*, 2009). This low amount of antirachitic activity demonstrates the importance of continuing infants on 400 IU/day even with maternal supplementation of 2,000 IU/day or 60,000 IU/month when baseline population vitamin D deficiency is so severe.

The inadequacy of 2,000 IU/day maternal intake to provide adequate vitamin D supply to the nursing infant also has been recently demonstrated in the United States. In a NIH-funded randomized, controlled trial of vitamin D dosing during lactation to support mother and infant, a study arm of 2,000 IU/day was terminated because this dose was 'inadequate' to supply sufficient vitamin D to maintain healthy vitamin D status of the breastfeeding infant (Hollis and Wagner, 2011). This study continues to investigate the safety and efficacy of 6,400 IU/day maternal intake and is near completion (Wagner *et al.*, 2006). This study is the next step in establishing the maternal vitamin D intake or status to adequately support the vitamin D health of the breastfed infant.

32.8 Conclusion

The resurgence of rickets described in case series and the high prevalence of infant vitamin D deficiency described in cross-sectional studies illustrate the global need for improvement not only in maternal and infant vitamin D recommendations, but also improvement in guaranteeing that the recommendations are followed. For those who are reticent to provide direct infant vitamin D supplementation due to belief that breast milk as the physiological gold-standard of infant nutrition could not be inadequate in a key nutrient, recognition that maternal vitamin D supplementation can support infant and maternal health while avoiding maternal toxicity is revolutionary.

Until the maternal dose for optimal safety and efficacy is established, 400 IU/day appears to be the infant intake associated with avoiding vitamin D deficiency rickets. However, research still is needed to identify the optimal vitamin D status for bone health, calcium homeostasis, and other vitamin D functions such as autoimmune, anti-infectious, and respiratory health. Moreover, further evaluation of the long-term effects of infant vitamin D status is required.

Until these studies are performed, ensuring vitamin D health of the mother/infant breastfeeding dyad includes recognition of the global prevalence of vitamin D deficiency, recognition of risk factors for vitamin D deficiency, including deficiency in pregnancy, and recommending at least 600 IU/day and potentially up to 4,000 IU/day maternal intake and 400 IU/day infant intake. With attention to these details and forthcoming study results, vitamin D deficiency rickets will again become a disease of the past.

References

Agarwal, N., Faridi, M.M., Aggarwal, A. and Singh, O., 2010. Vitamin D Status of term exclusively breastfed infants and their mothers from India. Acta Paediatrica 99, 1671-1674.

Ala-Houhala, M., Koskinen, T., Terho, A., Koivula, T. and Visakorpi, J., 1986. Maternal compared with infant vitamin D supplementation. Archives of Disease in Childhood 61, 1159-1163.

Ardeshirpour, L., Brian, S., Dann, P., VanHouten, J. and Wysolmerski, J., 2010. Increased PTHrP and decreased estrogens alter bone turnover but do not reproduce the full effects of lactation on the skeleton. Endocrinology 151, 5591-5601.

Balasubramanian, S., Shivbalan, S. and Kumar, P.S., 2006. Hypocalcemia due to vitamin D deficiency in exclusively breastfed infants. Indian Pediatrics 43, 247-251.

Basile, L.A., Taylor, S.N., Wagner, C.L., Horst, R.L. and Hollis, B.W., 2006. The effect of high-dose vitamin D supplementation on serum vitamin D levels and milk calcium concentration in lactating women and their infants. Breastfeeding Medicine 1, 27-35.

Basile, L.A., Taylor, S.N., Wagner, C.L., Quinones, L. and Hollis, B.W., 2007. Neonatal vitamin D status at birth at latitude 32 degrees 72': evidence of deficiency. Journal of Perinatology 27, 568-571.

Bhalala, U., Desai, M., Parekh, P., Mokal, R. and Chheda, B., 2007. Subclinical hypovitaminosis D among exclusively breastfed young infants. Indian Pediatrics 44, 897-901.

Challa, A., Ntourntoufi, A., Cholevas, V., Bitsori, M., Galanakis, E. and Andronikou, S., 2005. Breastfeeding and vitamin D status in Greece during the first 6 months of life. European Journal of Pediatrics 164, 724-729.

Davenport, M.L., Uckun, A. and Calikoglu, A.S., 2004. Pediatrician patterns of prescribing vitamin supplementation for infants: do they contribute to rickets? Pediatrics 113, 179-180.

Dawodu, A., Agarwal, M., Hossain, M., Kochiyil, J. and Zayed, R., 2003. Hypovitaminosis D and vitamin D deficiency in exclusively breast-feeding infants and their mothers in summer: a justification for vitamin D supplementation of breast-feeding infants. Journal of Pediatrics 142, 169-173.

Dawodu, A. and Tsang, R., 2007. Vitamin D deficiency and rickets: possible role of maternal vitamin D deficiency. Annals of Tropical Paediatrics 27, 319.

Dunn, P.M., 1998. Francis Glisson (1597-1677) and the "discovery" of rickets. Archives of Disease in Childhood: Fetal and Neonatal Edition 78, F154-155.

Fairweather-Tait, S., Prentice, A., Heumann, K.G., Jarjou, L.M., Stirling, D.M., Wharf, S.G. and Turnlund, J.R., 1995. Effect of calcium supplements and stage of lactation on the calcium absorption efficiency of lactating women accustomed to low calcium intakes. American Journal of Clinical Nutrition 62, 1188-1192.

Friedman, W.F., 1967. Vitamin D as a cause of the supravalvular aortic stenosis syndrome. American Heart Journal 73, 718-720.

Gartner, L.M. and Greer, F.R., 2003. Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. Pediatrics 111, 908-910.

Greer, F.R., Hollis, B.W., Cripps, D.J. and Tsang, R.C., 1984a. Effects of maternal ultraviolet B irradiation on vitamin D content of human milk. Journal of Pediatrics 105, 431-433.

Greer, F.R., Hollis, B.W. and Napoli, J.L., 1984b. High concentrations of vitamin D2 in human milk associated with pharmacologic doses of vitamin D2. Journal of Pediatrics 105, 61-64.

Greer, F.R. and Marshall, S., 1989. Bone mineral content, serum vitamin D metabolite concentrations, and ultraviolet B light exposure in infants fed human milk with and without vitamin D2 supplements. Journal of Pediatrics 114, 204-212.

Greer, F.R., Searcy, J.E., Levin, R.S., Steichen, J.J., Steichen-Asche, P.S. and Tsang, R.C., 1982. Bone mineral content and serum 25-hydroxyvitamin D concentrations in breast-fed infants with and without supplemental vitamin D: one-year follow-up. Journal of Pediatrics 100, 919-922.

Ho, M.L., Yen, H.C., Tsang, R.C., Specker, B.L., Chen, X.C. and Nichols, B.L., 1985. Randomized study of sunshine exposure and serum 25-OHD in breast-fed infants in Beijing, China. Journal of Pediatrics 107, 928-931.

Hollis, B.W., 1983. Individual quantitation of vitamin D2, vitamin D3, 25-hydroxyvitamin D2, and 25-hydroxyvitamin D3 in human milk. Analytical Biochemistry 131, 211-219.

Hollis, B.W., Roos, B.A., Draper, H.H. and Lambert, P.W., 1981. Vitamin D and its metabolites in human and bovine milk. Journal of Nutrition 111, 1240-1248.

Hollis, B.W. and Wagner, C.L., 2004. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. American Journal of Clinical Nutrition 80, 1752S-1758S.

Hollis, B.W. and Wagner, C.L., 2011. The vitamin D requirement during human lactation: the facts and IOM's 'utter' failure. Public Health Nutrition 14, 748-749.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R. and Virtanen, S.M., 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358, 1500-1503.

Institute of Medicine (IOM), 1997. Vitamin D, dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academy Press, Washington, DC, USA, pp. 250-287.

Jain, V., Gupta, N., Kalaivani, M., Jain, A., Sinha, A. and Agarwal, R., 2011. Vitamin D deficiency in healthy breastfed term infants at 3 months & their mothers in India: seasonal variation & determinants. Indian Journal of Medical Research 133, 267-273.

Kalkwarf, H.J. and Specker, B.L., 2002. Bone mineral changes during pregnancy and lactation. Endocrine 17, 49-53.

Karatekin, G., Kaya, A., Salihoglu, O., Balci, H. and Nuhoglu, A., 2009. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. European Journal of Clinical Nutrition 63, 473-477.

Kent, G.N., Price, R.I., Gutteridge, D.H., Smith, M., Allen, J.R., Bhagat, C.I., Barnes, M.P., Hickling, C.J., Retallack, R.W., Wilson, S.G. and *et al.*, 1990. Human lactation: forearm trabecular bone loss, increased bone turnover, and renal conservation of calcium and inorganic phosphate with recovery of bone mass following weaning. Journal of Bone and Mineral Research 5, 361-369.

Kim, M.J., Na, B., No, S.J., Han, H.S., Jeong, E.H., Lee, W., Han, Y. and Hyeun, T., 2010. Nutritional status of vitamin D and the effect of vitamin D supplementation in Korean breast-fed infants. Journal of Korean Medical Science 25, 83-89.

Kovacs, C.S., 2008. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. American Journal of Clinical Nutrition 88, 520S-528S.

Kovacs, C.S., 2011. Calcium and bone metabolism disorders during pregnancy and lactation. Endocrinology and Metabolism Clinics of North America 40, 795-826.

Kreiter, S.R., Schwartz, R.P., Kirkman Jr., H.N., Charlton, P.A., Calikoglu, A.S. and Davenport, M.L., 2000. Nutritional rickets in African American breast-fed infants. Journal of Pediatrics 137, 153-157.

Kumar, J., Muntner, P., Kaskel, F.J., Hailpern, S.M. and Melamed, M.L., 2009. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. Pediatrics 124, e362-370.

Madar, A.A., Stene, L.C. and Meyer, H.E., 2009. Vitamin D status among immigrant mothers from Pakistan, Turkey and Somalia and their infants attending child health clinics in Norway. British Journal of Nutrition 101, 1052-1058.

Maiya, S., Sullivan, I., Allgrove, J., Yates, R., Malone, M., Brain, C., Archer, N., Mok, Q., Daubeney, P., Tulloh, R. and Burch, M., 2008. Hypocalcaemia and vitamin D deficiency: an important, but preventable, cause of life-threatening infant heart failure. Heart 94, 581-584.

McNally, J.D., Leis, K., Matheson, L.A., Karuananyake, C., Sankaran, K. and Rosenberg, A.M., 2009. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatric Pulmonology 44, 981-988.

Morris, C.A. and Mervis, C.B., 2000. Williams syndrome and related disorders. Annu Rev Genomics Hum Genet 1, 461-484.

Park, M.J., Namgung, R., Kim, D.H. and Tsang, R.C., 1998. Bone mineral content is not reduced despite low vitamin D status in breast milk-fed infants versus cow's milk based formula-fed infants. Journal of Pediatrics 132, 641-645.

Polatti, F., Capuzzo, E., Viazzo, F., Colleoni, R. and Klersy, C., 1999. Bone mineral changes during and after lactation. Obstetrics and Gynecology 94, 52-56.

Reeve, L.E., Chesney, R.W. and DeLuca, H.F., 1982. Vitamin D of human milk: identification of biologically active forms. American Journal of Clinical Nutrition 36, 122-126.

Ross, A.C., Taylor, C.L., Yaktine, A.L. and Del Valle, H.B., 2011. DRI Dietary reference intakes calcium vitamin D. The National Academies Press, Washington, DC, USA, 1132 pp.

Roth, D.E., Jones, A.B., Prosser, C., Robinson, J.L. and Vohra, S., 2009. Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. European Journal of Clinical Nutrition 63, 297-299.

Roth, D.E., Shah, R., Black, R.E. and Baqui, A.H., 2010. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. Acta Paediatrica 99, 389-393.

Saadi, H.F., Dawodu, A., Afandi, B., Zayed, R., Benedict, S., Nagelkerke, N. and Hollis, B.W., 2009. Effect of combined maternal and infant vitamin D supplementation on vitamin D status of exclusively breastfed infants. Matern Child Nutr 5, 25-32.

Saadi, H.F., Dawodu, A., Afandi, B.O., Zayed, R., Benedict, S. and Nagelkerke, N., 2007. Efficacy of daily and monthly high-dose calciferol in vitamin D-deficient nulliparous and lactating women. American Journal of Clinical Nutrition 85, 1565-1571.

Sachan, A., Gupta, R., Das, V., Agarwal, A., Awasthi, P.K. and Bhatia, V., 2005. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. American Journal of Clinical Nutrition 81, 1060-1064.

Savino, F., Viola, S., Tarasco, V., Lupica, M.M., Castagno, E., Oggero, R. and Miniero, R., 2011. Bone mineral status in breast-fed infants: influence of vitamin D supplementation. European Journal of Clinical Nutrition 65, 335-339.

Seth, A., Marwaha, R.K., Singla, B., Aneja, S., Mehrotra, P., Sastry, A., Khurana, M.L., Mani, K., Sharma, B. and Tandon, N., 2009. Vitamin D nutritional status of exclusively breast fed infants and their mothers. Journal of Pediatric Endocrinology and Metabolism 22, 241-246.

Shaikh, U. and Alpert, P.T., 2004. Practices of vitamin D recommendation in Las Vegas, Nevada. Journal of Human Lactation 20, 56-61.

Sowers, M., 1996. Pregnancy and lactation as risk factors for subsequent bone loss and osteoporosis. Journal of Bone and Mineral Research 11, 1052-1060.

Sowers, M.F., Hollis, B.W., Shapiro, B., Randolph, J., Janney, C.A., Zhang, D., Schork, A., Crutchfield, M., Stanczyk, F. and Russell-Aulet, M., 1996. Elevated parathyroid hormone-related peptide associated with lactation and bone density loss. JAMA 276, 549-554.

Specker, B.L., Ho, M.L., Oestreich, A., Yin, T.A., Shui, Q.M., Chen, X.C. and Tsang, R.C., 1992. Prospective study of vitamin D supplementation and rickets in China. Journal of Pediatrics 120, 733-739.

Specker, B.L., Vieira, N.E., O'Brien, K.O., Ho, M.L., Heubi, J.E., Abrams, S.A. and Yergey, A.L., 1994. Calcium kinetics in lactating women with low and high calcium intakes. American Journal of Clinical Nutrition 59, 593-599.

Taussig, H.B., 1966. Possible injury to the cardiovascular system from vitamin D. Annals of Internal Medicine 65, 1195-1200.

Taylor, J.A., Geyer, L.J. and Feldman, K.W., 2010. Use of supplemental vitamin d among infants breastfed for prolonged periods. Pediatrics 125, 105-111.

Tomashek, K.M., Nesby, S., Scanlon, K.S., Cogswell, M.E., Powell, K.E., Parashar, U.D., Mellinger-Birdsong, A., Grummer-Strawn, L.M. and Dietz, W.H., 2001. Nutritional rickets in Georgia. Pediatrics 107, E45.

Wagner, C.L. and Greer, F.R., 2008. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 122, 1142-1152.

Wagner, C.L., Howard, C., Hulsey, T.C., Lawrence, R.A., Taylor, S.N., Will, H., Ebeling, M., Hutson, J. and Hollis, B.W., 2010. Circulating 25-hydroxyvitamin d levels in fully breastfed infants on oral vitamin d supplementation. International Journal of Endocrinology 2010, 235035.

Wagner, C.L., Hulsey, T.C., Fanning, D., Ebeling, M. and Hollis, B.W., 2006. High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. Breastfeeding Medicine 1, 59-70.

Wayse, V., Yousafzai, A., Mogale, K. and Filteau, S., 2004. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. European Journal of Clinical Nutrition 58, 563-567.

Weisman, Y., Bawnik, J.C., Eisenberg, Z. and Spirer, Z., 1982. Vitamin D metabolites in human milk. Journal of Pediatrics 100, 745-748.

Welch, T.R., Bergstrom, W.H. and Tsang, R.C., 2000. Vitamin D-deficient rickets: the reemergence of a once-conquered disease. Journal of Pediatrics 137, 143-145.

Zamora, S.A., Rizzoli, R., Belli, D.C., Slosman, D.O. and Bonjour, J.P., 1999. Vitamin D supplementation during infancy is associated with higher bone mineral mass in prepubertal girls. Journal of Clinical Endocrinology and Metabolism 84, 4541-4544.

Zeghoud, F., Vervel, C., Guillozo, H., Walrant-Debray, O., Boutignon, H. and Garabedian, M., 1997. Subclinical vitamin D deficiency in neonates: definition and response to vitamin D supplements. American Journal of Clinical Nutrition 65, 771-778.

Ziegler, E.E., Hollis, B.W., Nelson, S.E. and Jeter, J.M., 2006. Vitamin D deficiency in breastfed infants in Iowa. Pediatrics 118, 603-610.

Key facts

- Vitamin D level during pregnancy has been found to be surprisingly low in an appreciable proportion of women from developing countries, even from rural communities.
- Vitamin D fortified food is not widely available in developing countries, and pregnant women from a rural or poor socioeconomic class have inadequate dietary calcium intake.
- Supplementing vitamin D on a daily basis is a challenge in developing countries, where there is already poor utilization of antenatal services.
- Periodic interval supplementation that can be directly observed and given during the antenatal visit may hold promise for various developing countries.
- Providing a pharmacological dose of vitamin D during second and third trimester, which can be matched in timing with the minimal antenatal visits, has been shown to be effective in improving 25(OH)D levels at delivery and may beneficially affect neonatal anthropometry. A weekly regimen trial can also be used in some settings.

Summary points

- There is heterogeneity in prevalence data even at the same latitude, which reflects sunshine exposure and diet based on local culture and customs.
- Pregnant women in the third trimester with homebound activities and during winter constitute the most vulnerable group.
- Women from developing countries have a low dietary calcium intake. Lack of food fortification or lack of use of supplements leaves exposure to sunshine as the only source of vitamin D, a factor that may be inadequate in many settings despite abundant sunshine.
- Vitamin D supplementation during pregnancy needs attention in many developing regions such as South Asia, and the Middle East which have observed the maximum burden.
- Some studies have shown that it may affect the outcome of infection in mother and child during pregnancy, a topic relevant to developing countries.
- Weekly, monthly, or trimester specific supplementation as opposed to daily supplementation is a more promising prospect for pregnant women from developing countries in view of compliance.
- Against a background of minimal use of antenatal services, pharmacological dose supplementation can be offered at the same time as contact with health care providers during second and third trimester, providing the benefit of observed therapy.
- Long-term safety and efficacy data will need to be generated for intervention and supplementation studies.

33. Vitamin D replacement in pregnant women in developing countries

M.T. Sahu[1,2] and M. Sahu[3]
[1]ADIVA Hospital, 15 Anand Lok, New Delhi 110049, India; [2]Institute of Liver and Biliary Sciences, D-1, Vasant Kunj, New Delhi, India; [3]VIMHANS Hospital, No.1, Institutional Area, Nehru Nagar, New Delhi 11065, India; meenakshi_sahu@rediffmail.com

Abstract

Many pregnant women residing in developing countries have inadequate vitamin D levels despite the fact that some of these countries are at a lower latitude. Darker pigmentation with inadequate sun exposure, a home-bound lifestyle especially during the third trimester, and limited dietary source of vitamin D due to lack of availability of fortified foods, all contribute towards maternal hypovitaminosis D. Inadequate vitamin D levels further contribute to the precarious nutritional state accompanied by a markedly inadequate intake of dietary calcium typically observed in many low resource settings. Early marriage along with early pregnancy are some of the other challenges specific to developing parts and attention is needed for vitamin D and bone health issues during pregnancy. Some regions, especially South Asia, have noted a high prevalence of vitamin D insufficiency among pregnant women aggravated during the winter. Despite the high prevalence vitamin D insufficiency and the importance of vitamin D during pregnancy, it continues to remain an underappreciated problem in many developing countries. Infrequent and inadequate use of antenatal care by many rural women provides a limited window for assessing risk and planning intervention with measures in the area of education or medication. Daily vitamin D supplementation may meet challenges related to compliance in low resource settings. Pharmacological doses per week or per trimester have been observed to improve 25(OH)D levels at delivery and amidst inconsistent data some trials have shown a beneficial impact on the anthropometry of new-borns. There have been no reports of any adverse risk to mother or child with such an approach. There will continue to be a debate on what constitutes adequate levels and whether vitamin D supplementation indeed influences any maternal or fetal outcome. While one reaches to find conclusive answers, given the growing burden of this condition, periodic supplementation should be offered to pregnant females at risk.

Keywords: supplementation, low resource, rural, antenatal, maternal

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_33, © Wageningen Academic Publishers 2013

Abbreviations

25(OH)D	25-hydroxyvitamin D
VDD	Vitamin D deficiency
UVB	Ultraviolet B

33.1 Introduction

Many developing nations, despite several of them being at lower latitudes, have vitamin D deficiency among their populations (Arabi *et al.*, 2010; Van Schoor and Lips, 2011*)*. Recent hospital and community-based studies from these parts have observed vitamin D insufficiency among an appreciable proportion of females during their reproductive period. Given the advanced understanding of the multiple role of vitamin D in human health (Holick, 2007; Vieth, 2011), VDD during pregnancy has been a subject of great interest to many.

Maintaining an adequate level of vitamin D during pregnancy may play an important role such as ensuring adequacy of calcium for the growing fetus without any adverse effect on maternal bone health; reducing risk outcome such as preeclampsia, neonatal hypocalcaemia and infections; a likely effect on neonate anthropometry; and a possible role in fetal programming of long-term skeletal and non-skeletal health benefits (Barrett and McElduff, 2010; De-Regil *et al.*, 2012; Dror and Allen, 2010; Hollis and Wagner, 2011; Thandrayen and Pettifor, 2010). The general impact of maternal vitamin D levels on pregnancy and neonatal outcome is discussed in Chapter 31.

Specific challenges in developing countries are the markedly low intake of dietary calcium, lack of availability of fortified foods, early marriage, adolescent pregnancy, and barriers to using a modern health care delivery system. With all the above background, it is important for the individual practitioner and health policy makers in developing nations to be aware of their geographical prevalence data and take pragmatic measures to replace vitamin D in pregnant women.

This chapter provides a review of vitamin D deficiency and its replacement in pregnant women from developing nations. For the purposes of the current chapter, developing nations were considered as per World Bank classification (World Bank, 2012). The essence of chapter is for poor resource setting with its challenges in reproductive health care such as what exists in a vast majority of rural population at many developing countries.

33.2 Hypovitaminosis D during pregnancy: magnitude and relevance to developing countries

33.2.1 Causative factors

Vitamin D deficiency and its implications during pregnancy have long been recognized from developing parts of the world (Maxwell and Miles, 1925). Multiple past and recent studies have shown vulnerability to this condition among immigrants from developing countries (Bashir *et al.*, 1981; Hodgkin *et al.*, 1973; Madar *et al.*, 2009; Van der Meer *et al.*, 2006; Watney *et al.*, 1971). Some of the reasons cited for immigrants are factors such as dark complexion, cultural dress that restricts exposure of skin to the sun, and low intake of supplements.

Most of these factors observed among immigrant women have also been observed to exist in antenatal women residing in their native country. Darker pigmented skin needs more exposure to UVB rays than fair pigmented skin to generate the same amount of vitamin D (Gemens *et al.*, 1982; Holick *et al.*, 1981; Lo *et al.*, 1986; Matsuoka *et al.*, 1991). It is being realized that despite many of the developing nations having year round sunshine, the amount of time that a woman spends outdoor may be shorter than that required for adequate levels (Sachan *et al.*, 2005; Sahu *et al.*, 2009a). While the practice of purdah (use of veils) is known to affect vitamin D levels, cultural dress such as the *sari* in some South Asian countries (the dress usually leaves only head and arms exposed) also limits exposure to sunshine (Islam *et al.*, 2002; Sachan *et al.*, 2005; Sahu *et al.*, 2009a). Furthermore, women avoid outdoor activities during the time of peak solar energy (noon time), and pregnant females during their third trimester are restricted to indoor activities. Being non-veiled does not protect women from VDD compared to veiled women if there is a homebound lifestyle and only occasional sun exposure (Islam *et al.*, 2006). A growing index of atmospheric pollution in urban settings as the cost of rapid progress in developing countries can limit the amount of UVB solar radiation reaching the ground and may also contribute to VDD (Agarwal *et al.*, 2002). Although it merits detailed study in rural settings, atmospheric brown clouds due to aerosol emanating from biofuels that is used for domestic purposes in rural regions (Di Girolamo *et al.*, 2004; Ramanathan *et al.*, 2005) can scatter solar radiation, and may have a role to play.

33.2.2 Characteristics of developing countries

Within the context of vitamin D and pregnancy, two important factors that differentiate high and low income countries, is firstly the low intake of dietary calcium, and secondly the lack of availability of vitamin D fortified foods (Prentice, 2011). Consumption of animal milk and milk products is low in many African and Asian countries compared to the developed world (Food and Agriculture Organization, 2001). Furthermore, pregnant women from rural regions usually have a monotonous cereal-based diet high in phytates that can further decrease bioavailability of calcium (Cheng *et al.*, 2009; Panwar and Punia, 2000). Most developing countries have yet to witness wide access to vitamin D fortified foods. Thus, many pregnant females are not only on a diet that is markedly low in calcium (sometimes as low as 200 mg per day), but are also deprived

of adequate levels of vitamin D due to the above factors. Interestingly, low calcium intake has been hypothesized as leading to secondary vitamin D deficiency (Clements *et al.*, 1987; Harinarayan *et al.*, 2007; Islam *et al.*, 2002; Sachan *et al.*, 2005; Sahu *et al.*, 2009a). While in a state of vitamin D and calcium deprivation, maternal physiology will adapt to ensure that the fetus gets the calcium, but this comes at the expense of the mothers, putting them at risk of osteomalacia and other adverse outcomes (Barrett and McElduff, 2010; Prentice, 2011).

Early childhood marriage is still a prevalent practice in many developing countries, mostly observed in Africa and Asia, with some regions such as Nigeria, Bangladesh having more than half of girls married before the age of 18 (Population Reference Bureau, 2011). In addition, many developing regions have reported a high prevalence of vitamin D deficiency among adolescent girls (Du *et al.*, 2001; Sahu *et al.*, 2009a). It is to be presumed that many such adolescent girls enter pregnancy with an already depleted store of vitamin D and a poor skeletal mineral mass. Untreated osteomalacia from a young age can affect pelvic outlet that may impact the route of delivery (Brickley and Ives, 2008; Kabakyenga *et al.*, 2011; Konje and Ladipo, 2000). It is also our anecdotal observation that pregnant women from poor economic strata who present with obstructed labor due to an abnormal pelvic outlet have been in a state of impoverished nutrition since childhood and adolescence, and have usually undergone early marriage and pregnancy, with a clinical picture suggestive of clinical osteomalacia (bone and muscle pain, muscle weakness) in previous pregnancies. It is possible that, if left untreated, each pregnancy with low nutrition status starting from adolescence can impact the outcome of successive pregnancies. Such an observation, however, remains unproven. There have been conflicting reports supporting (Merewood *et al.*, 2009) or refuting (Brunvand *et al.*, 1998) association of vitamin D status at time of delivery to route of delivery. It is essential that the focus is placed on bone health during adolescence and pregnancy, as adolescent pregnancy is a predictor for post-menopausal osteoporosis (Cho *et al.*, 2012).

Low birth weight is a challenging issue in developing countries with its associated risk of morbidity and mortality (McIntire *et al.*, 1999). Newborn size, usually a reflection of prenatal growth, can be affected by maternal nutritional status. Although overall data on vitamin D and its effect on offspring size is inconsistent (Specker, 2012), a few have demonstrated a correlation between neonatal anthropometry and cord blood calcium (Doi *et al.*, 2011) or vitamin D sufficiency in mothers (Kalra *et al.*, 2012; Lefelaar *et al.*, 2010).

Other characteristics in the context of supplementation during pregnancy are provided in the section on supplementation.

33.2.3 Magnitude and impact

The prevalence of vitamin D insufficiency among pregnant females reported in selective studies from developing parts of the world is provided in Table 33.1. While most have utilized the cut-off of 25 nmol/l to define deficiency, there have been debates on what constitutes a sufficient level. Some suggest using levels of 75 nmol/l while others have advocated using 50 nmol/l for

Table 33.1. Prevalence of hypovitaminosis D in pregnant women from developing countries.

Region/country	Latitude	n	Prevalence (%)	Cut-off (nmol/l)	Mean value (nmol/l)	Comments
Sub-Saharan Africa						
Gambia						
Prentice *et al.*, 2009a	13°N	125	16	<80	111±27	Pregnant women from rural villages. Data shown here is at 36th week of gestation. Data is a subset from a calcium intervention study
			None	<50		
Ethiopia						
Feleke *et al.*, 1999	10°N	31	81	<50	25 (17-46)[a]	Full term pregnant women. Sample collected during August and September
			55	<30		
Tanzania						
Mehta *et al.*, 2010	6°S	884	40	<80	89 ±31	HIV infected pregnant women at 12-27th week. Data is a subset of baseline data from a vitamin trial supplementation study
Middle East and North Africa						
Iran						
Maghbooli *et al.*, 2007	35°N	552	97	<80	27.8±21.71	Recruited during winter season
			67	<35		
Bassir *et al.*, 2001	35°N	50	80	<25	12.8±26	Women who delivered between January and September were recruited
Latin America & Caribbean						
Argentina						
Oliveri *et al.*, 1993	55°S	16	62	<20	15.8±12	16 pregnant women from Ushuaia (55°S) and 21 pregnant women from Buenos Aires (34°S). Study conducted at end of winter
	34°S	21	24		36±21	

Table 33.1. Continued.

Region/country	Latitude	n	Prevalence (%)	Cut-off (nmol/l)	Mean value (nmol/l)	Comments
East Asia and Pacific						
China						
Wang *et al.*, 2010	26-34 °N	77	97	<80	35.9±19.7	West China. September recruitment. Significantly low calcium intake observed in rural women as compared to urban women. Lower vitamin D in rural women compared to urban
			57	<37.5		
Jiang *et al.*, 2012	31°N	152	94.7	<50	31.9±9.2	South East China. Urban residents. Data shown here collected in summer. Vitamin D levels were significantly lower in winter
Europe and Central Asia						
Turkey						
Ergür *et al.*, 2009	39.5°N	70	81	<62.5	39.8 (10-125.5)[a]	Sample collected during the first 24 hours after delivery in mothers. Recruitment over two years
			27	<27.5		
Halicioglu *et al.*, 2012	38.3°N	258	90	<50	27.5±13.5	Last trimester sample. Half of these women had covered dressing style. 58% did not use any multivitamin. Study period was spring time: March, April, May
			50	<25		
South Asia						
India						
Marwaha *et al.*, 2011	28.4°N	541	96	<50	23.2±12.2	Females from lower middle socioeconomic strata. Samples obtained from all trimesters. No significant differences across the trimesters were noticed
			60	<25		
Farrant *et al.*, 2009	12.2°N	559	66	<50	37.8 (24.0-58.5)[a]	25(OH)D assessed at 30 weeks gestation. 156 participants were taking calcium and vitamin D at recruitment
			31	<28		
Sachan *et al.*, 2005	26.8°N	207	84	<56.3	35.3±22.3 (rural)	PTH rose above range when 25(OH)D was less than 56.3 nmol/l. 14% mothers had elevated HLAP [b]. 25(OH)D levels similar in urban and rural
			67	<37.5		
			43	<25		
Sahu *et al.*, 2009a	26.8°N	139	74	<50	37.8±19.8	Rural community prevalence data. 43% had raised HLAP [b]. Mean dietary calcium intake was 214 mg per day
			32	<25		

Table 33.1. Continued.

Region/country	Latitude	n	Prevalence (%)	Cut-off (nmol/l)	Mean value (nmol/l)	Comments
South Asia (continued)						
Bangladesh						
Islam *et al.*, 2002	24.5°N	99 rural	50	<37.5	44.8±20	Comparative study between low (rural) and high socioeconomic region (n=99 and 90 respectively). Study population comprised pregnant, non-pregnant and lactating women. No difference in 25(OH)D levels between low or high socioeconomic status
			17	<25		
Pakistan						
Hossain *et al.*, 2011	24.5°N	75	89	<75	32.9±16.9	Pregnant women at term. During summer
Karim *et al.*, 2011	24.5°N	50	78	<75	60	Sample from patients admitted in labor
			46	<50		
Nepal						
Jiang *et al.*, 2005	27°N	1,163	14 (all)			
			24.4 (winter)	<25	51.1	Subset data from population-based maternal micronutrient supplementation trial Study carried out at rural Nepal. First trimester enrollment

[a] Median values reported.

[b] Heat labile alkaline phosphatase.

defining sufficiency (Holick, 2007; Holick *et al.*, 2011; Institute of Medicine, 2011; Lips, 2007). Nevertheless, overall data reveals that vitamin D insufficiency exists at an unexpectedly high rate in some of these countries even by the definition of 50 nmol/l.

Most of the data on VDD during pregnancy has been obtained from hospital or community-based studies from South Asia. Many other regions have yet to generate data. In the absence of local data, a clue to the burden of this condition during pregnancy should be obtained from existing studies performed on non-pregnant females in their reproductive age group. The old universal statement that vitamin D insufficiency during pregnancy is not an issue in developing countries as many of the women live at low lying latitude, is not necessarily correct. It is hard to predict the magnitude of this condition based on latitude, as the prevalence data is markedly heterogeneous between studies from similar latitudes. As an example, in a study of south-east Asian women of child-bearing age group (18-40 years) residing near the equator at Jakarta (6°S) and Kuala Lumpur (2°N), over 60% of participants had insufficient 25(OH)D <50 nmol/l (Green *et al.*, 2008). A study from Fiji (18°S) showed that among females in the reproductive age group (15-44 years) mostly from rural communities, approximately half of the participants had 25(OH) D less than 80 nmol/l, with 11% of all women participants at a level below 50 nmol/l (Heere *et al.*, 2010). The mean 25(OH)D from Fijian Indians from this study was 70 nmol/l, with 21% having 25(OH)D levels less than 50 nmol/l. In contrast, rural women from South India (latitude 13.4 °N) demonstrated a mean of 47.5 nmol/l with 70% of them having 25(OH)D levels less than 50 nmol/l (Harinarayan *et al.*, 2007). To add to such heterogeneity, observations in pregnant women from rural areas of The Gambia, West Africa (13°N) revealed that none of the pregnant females participating in a study had 25(OH)D value <50 nmol/l (Prentice *et al.*, 2009a). What may explain such wide variation is the actual duration of sunshine exposure, as some have noticed that women spend significant time outdoors (Prentice *et al.*, 2009a) while other studies have suggested factors such as pigmentation, dress, and deliberate avoidance of sunshine during peak solar energy time (Islam *et al.*, 2002; Sahu *et al.*, 2009a). All these studies only reinforce the notion that the cheapest abundant source of vitamin D (sunshine!) goes underutilized in many rural areas as well.

Predisposition to extremely low levels of vitamin D has been noted in winter despite the fact that some of these regions have abundant solar energy year round (Ergür *et al.*, 2009; Maghbooli *et al.*, 2007; Marwaha *et al.*, 2011; Namgung *et al.*, 1998; Sahu *et al.*, 2009a). Foggy winters and the monsoon season are vulnerable periods that can limit the amount of UVB solar energy reaching the ground's surface. Because calcium accretion occurs at maximal rates during the third trimester (Prentice, 2011), homebound pregnant women in their last trimester during winter are the most vulnerable population. Coexistent infection in mothers, prevalent in developing regions, also constitutes a risk. Observations from Tanzania (6°S) suggest that vitamin D status at 12-27 weeks of gestation in HIV-infected mothers affects mother-to-child transmission and child mortality. Approximately 39% of pregnant women (347 out of 885) had vitamin D levels <80 nmol/l. Infants born to mothers who had low levels of vitamin D had a 49% higher risk of dying or being HIV-infected at birth and were more likely to report cough during follow-up along with an increased risk of stunting and being underweight (Finkelstein *et al.*, 2012; Mehta *et al.*, 2009).

In summary, many pregnant women from developing countries, even from rural regions, have insufficient 25(OH)D levels. Specific issues relevant to developing countries are low intake of calcium among pregnant women; early marriage and adolescent pregnancy with its adverse effect on bone health if calcium intake and vitamin D status are inadequate; higher incidence of low birth weight; predisposition to maternal and neonatal infection; and less contact made with health care delivery system (see Section 33.4). Despite the controversy about what constitutes adequate vitamin D levels during pregnancy, there are vast numbers of females residing in developing countries who would benefit from vitamin D supplementation.

33.3 Vitamin D supplementation

Some suggest that the daily dosage required by pregnant women may be considerably higher than the usual 400 IU available in most supplements or higher than the 600 IU recommended dietary allowance or actually be as high as 4,000 IU daily (Holick *et al.*, 2011; Hollis and Wagner, 2011; Hollis *et al.*, 2011). Others have questioned the utility of and need for more vitamin D than the usual 400 to 600 IU daily (Abrams, 2011; Rosen *et al.*, 2012). While the debate goes on, it needs to be emphasized that pregnant women in developed countries take either vitamin D fortified foods or supplements that contain vitamin D, a scenario that does not exist in many developing parts of the world. Moreover, as noted in the preceding section, an appreciable proportion of pregnant women (in some places 50-90%) from developing countries do not have sufficient vitamin D levels of 50 nmol/l and many (in some places one third to a half) have vitamin D levels less than 25 nmol/l.

In the context of vitamin D supplementation, one of the limitations felt to be characteristic for low resource settings is a lack of diagnostic tools to check and monitor 25(OH)D levels as well as monitor any adverse effects caused by supplementation in pregnant women who spend many hours in the sun. Secondly, although the utilization of antenatal services has increased in recent years in developing nations, the rural population with poor educational status and living in poverty still remain neglected and have infrequent contact with the antenatal health care system (Barros *et al.*, 2012; Pallikadavath *et al.*, 2004; WHO, 2009). Compliance with daily supplementation can thus be challenging given such infrequent contact. There are some solutions to the above challenges: firstly, the lack of adverse effects in humans reported from interventional studies including trials using a daily dose of 4,000 IU provide some comfort for low resource settings without tools for monitoring. Secondly, to enhance compliance, a weekly or monthly pharmacological dose (stoss) therapy during each trimester is a practical alternative, with the latter having the added advantage of being combined with the limited antenatal health care visits.

Very few intervention studies have been conducted in hospital or community-based settings from developing countries.

Pregnant Asian immigrant women in the UK receiving ergocalciferol 1000 units/ day during the last trimester experienced a significant elevation of their vitamin D levels at term, with a

mean value of 168 nmol/l compared to baseline level of 20 nmol/l (Brooke *et al.*, 1980). Of clinical importance is the observation that none of the infants born to mothers who took supplementation developed symptomatic hypercalcaemia. Though there was no significant effect on anthropometry immediately after birth, Brooke *et al.* (1981) later demonstrated improved post-natal growth at one year in infants whose mothers received vitamin D during pregnancy. Outwardly it may seem that 1000 units per day is good enough for pregnant Asians in view of the rise in 25(OH)D at term, but another study with a similar ethnic group showed that on giving calciferol 800 IU per day, 25(OH)D levels rose from 14.9 nmol/l to only 27.5 nmol/l (Datta *et al.*, 2002). Detailed analyses suggest that the above-mentioned study that used ergocalciferol 1000 IU per day may have contained a higher dose than was indicated on the label, hence the anomolous prominent response in raising 25(OH)D levels (Hollis and Wagner, 2004). In contrast to no significant effect on birth anthropometry by daily supplementation (Brooke *et al.*, 1980), a study carried out in India reported significant improvements in the anthropometry of newborns in women who received a pharmacological dose of 600,000 IU each in the seventh and eighth month of pregnancy, without any reports of hypercalcemia (Marya *et al.*, 1988). Vitamin D was not reported from this study. The improvement in birth weight and maternal weight was speculated to be partly due to better maternal nutrition after the removal of ill-defined anorexia due to hypovitaminosis D (Brooke *et al.*, 1980; Marya *et al.*, 1988).

In a pilot intervention study carried out in a rural community setting in India (Sahu *et al.*, 2009b), 84 women belonging to a poor socioeconomic class were compared after providing them with either education about sun exposure, 60,000 IU of cholecalciferol during the second trimester, or 120,000 IU each in the fifth and seventh month. In the group that received mega doses of 120,000 IU during the second and third trimester, there was a significant elevation in 25(OH)D levels post-delivery as compared to baseline. However, even with this regimen only 34% of women attained 25(OH)D levels more than 80 nmol/l. There was no documentation of hypervitaminosis D (more than 375 nmol/l) in any of the subjects. Education on sunlight exposure did not lead to any further behavioral change. Although the 25(OH)D levels at delivery may not reflect the dose effect of cholecalciferol if given three months before measurement (Ilahi *et al.*, 2008), the regimen selected was a practical measure to coincide with the minimal once per trimester antenatal visit in rural areas.

As part of the next step of assessment, at the same latitude and using the same dose regimen, 97 pregnant females of lower middle and middle socioeconomic status were followed up in a hospital setting and observed for maternal 25(OH)D levels and newborn and infant anthropometry (Kalra *et al.*, 2012). Maternal 25(OH)D levels at term were significantly higher in the group that received 120,000 IU of vitamin D during the second and third trimester, with 62% of these women having 25(OH)D more than 50 nmol/l at delivery. Significantly better anthropometry in the newborn was observed in those mothers who received either regimen, as compared to a control group. The better biophysical profile continued to remain till nine months of follow-up. These findings acquire further significance in the light of a study report in which it was observed that vitamin D status during late pregnancy correlates to whole body bone mineral content in children at age 9 years (Javaid *et al.*, 2006).

Weekly dose has also been recently studied in Bangladesh. Non-pregnant and pregnant women between the 26th and 30th week of gestation were enrolled and observed with either a single dose 70,000 IU or a weekly dose of 35,000 IU in a preliminary dose finding and safety study (clinicaltrials.gov identifier: NCT00938600). Unpublished data suggest that using 35,000 IU per week in the third trimester showed no study drug related adverse event (Roth, 2011). At the time of writing this chapter, a trial subsequent to the dose-finding study is underway in Bangladesh and is studying the effect of 35,000 IU per week starting from the 26th to 29th week of gestation on the outcome of maternal and infant vitamin D status and neonatal immune function (clinicaltrials.gov identifier: NCT01126528).

Given the fact that there is a limited dietary source of vitamin D, a daily minimum dose is also a valuable alternative for pregnant women, if compliance allows, and it should be advocated at a bare minimum. In a nutrient intervention trial conducted in rural Nepal, 400 IU of vitamin D as a component of multiple micronutrient supplements was provided from the first trimester onwards. Although it was not a study designed to observe the effect of vitamin D supplementation, data from this trial suggest that the intake of 400 IU of vitamin D daily from the first trimester onwards is beneficial in modestly raising 25(OH)D levels with a change in mean value of 17.8 nmol/l from a baseline of 47.4±23.5 nmol/l (Christian *et al.*, 2006). A recent report from an elegant randomized study conducted at latitude 32°N in the USA, has shown that taking 400 IU daily from the first trimester leads to changes in circulating 25(OH)D levels around delivery. However, it was only in pregnant women taking 2,000 or 4,000 IU daily that the vast majority (93% and 94%, respectively, compared to 57% in 400 IU daily) achieved 25(OH)D levels more than 80 nmol/l one month prior to or at delivery There was no difference observed between maternal and fetal outcome at time of delivery between the different dosage (Hollis *et al.*, 2011).

What should be the best regimen for pregnant women in low resource settings? In the absence of large-scale data from such areas, the choice of treatment should be individualized for either daily, monthly or trimester specific therapy based on setting and anticipated compliance. There are only a few comparative studies between the daily or periodic pharmacological dose of vitamin D during pregnancy to help provide an answer. In a comparative study from the UK, daily supplementation of 800 IU daily and a single oral dose supplementation of 200,000 IU beginning from the 27th week of gestation showed comparable results, however only 30% of the women achieved vitamin D sufficiency (>50 nmol/l) at term (Yu *et al.*, 2009). Similar findings were also reported, albeit in lactating women, who received a daily ergocalciferol dose of 2,000 IU daily or a monthly dose of 60,000 IU for three months, with both being effective. Only 35% in the daily group and 20% in the monthly group attained vitamin D sufficiency after three months of use (Saadi *et al.*, 2007).

The debate will continue to run on what constitutes an efficacious and safe regimen of vitamin D supplementation (IOM, 2011; Roth, 2011), and whether some developing countries should focus on this aspect, especially if some regions have not revealed any significant burden of vitamin D deficiency (Prentice *et al.*, 2009b). With the current knowledge on the widespread prevalence of vitamin D insufficiency especially in some parts of the world such as the Asian region, it does not seem prudent to withhold vitamin D supplementation in these areas. Even in regions

near the equator, the adequacy of vitamin D levels during pregnancy is not widespread (see section on magnitude above). At present, the use of intermittent high dose (stoss) therapy in low resource settings appears to be convenient, inexpensive, practical, and without any reports of hypercalcemia from the limited intervention studies available. It should be noted, however, that most of the high dose supplementation studies have been carried out during the second or third trimester (Kalra *et al.*, 2012; Madelenat *et al.*, 2001; Roth, 2011; Sahu *et al.*, 2009b). Thus, pharmacological stoss therapy should not be attempted during the first trimester where safety has not been established. The use of parenteral cholecalciferol (available in doses of three to six lac units in some countries) should be avoided during pregnancy despite limited data on efficacy and safety in post-partum women (Mozaffari-Khosravi *et al.*, 2012).

In summary, though debatable and subjective, based on current data from low resource settings, it appears that more than the recommended 400-600 IU of vitamin D daily is required during pregnancy to achieve satisfactory blood levels of 25(OH)D at term. In the absence of large-scale randomized data from safety and health outcome data in infants, in settings where vitamin D deficiency cannot be diagnosed, a minimum dose of 60,000 IU of vitamin D given once in the middle of the second and third trimester seems promising without generating excessive concerns about safety. As seen across some trials, this dose can be conservative and may lead to only modest elevation of vitamin D levels. Thus, providing an additional dose of 60,000 IU vitamin D during the second and third trimester apart from the above regimen, especially during the winter, may prove beneficial. In the absence of elaborate safety data during pregnancy, we suggest at least a month's interval between each pharmacological dosing. Consideration should also be given to this higher dosing regimen if a pregnant woman presents with a clinical picture suggestive of osteomalacia. As an alternative to the high-dose therapy, if compliance can be ensured, daily supplementation of vitamin D with at least 600 to 1000 IU daily from the second trimester or a higher dose of 2,000 IU can be offered. However, daily supplements at this dose may be unavailable in many regions, and there may also be compliance problems. Lastly, it makes no sense to focus purely on vitamin D supplementation without reinforcing the importance of a calcium-rich diet or calcium supplements, the intake of which is very low among pregnant women in many rural areas.

33.4 Areas of uncertainty and progress

Though there has been an assurance that no cases of maternal hypervitaminosis D have emerged during vitamin D supplementation trials in pregnancy, there is still no long-term safety follow-up in children whose mothers received mega doses of vitamin D as opposed to a daily dose. There a few trials in progress at the time of writing this chapter, and detailed reports may shed some more light on the risk-benefit ratio of supplementation (Harvey *et al.*, 2012; Roth, 2011). While the magnitude of VDD during pregnancy is being appreciated as per new studies, reports on the beneficial health outcome of pregnancy have been inconsistent (De-Regil *et al.*, 2012; Specker, 2012). Nevertheless, this does not diminish the fact that in some regions of developing countries,

even in rural areas, there are appreciable numbers of pregnant women who suffer from vitamin D deficiency and would benefit from vitamin D replacement.

33.5 Conclusions

In conclusion, vitamin D deficiency in pregnant women is an under-recognized problem among many obstetricians and health care providers despite the high prevalence of this condition in some developing nations. The absence of food fortification programs, low calcium intake during pregnancy, issues of adolescent pregnancy, and limited utilization of antenatal care, makes pregnant women from developing countries more vulnerable and in need of attention and care in the context of vitamin D, calcium and skeletal health. Periodic vitamin D supplementation as opposed to daily supplementation during pregnancy, starting from the second trimester, seems a practical measure in settings with limited resources.

References

Abrams, S.A., 2011. Vitamin D supplementation during pregnancy. J Bone Miner Res 26, 2338-2340.

Agarwal, K.S., Mughal, M.Z., Upadhyay, P., Berry, J.L., Mawer, E.B. and Puliyel, J.M., 2002. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. Arch Dis Child 87, 111-113.

Arabi, A., El Rassi, R. and El-Hajj Fuleihan, G., 2010. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nat Rev Endocrinol 6, 550-561.

Barrett, H. and McElduff, A., 2010. Vitamin D and pregnancy: an old problem revisited. Best Pract Res Clin Endocrinol Metab 24, 527-539.

Barros, A.J., Ronsmans, C., Axelson, H., Loaiza. E., Bertoldi, A.D., França, G.V., Bryce, J., Boerma, J.T. and Victora, C.G., 2012. Equity in maternal, newborn, and child health interventions in Countdown to 2015: a retrospective review of survey data from 54 countries. Lancet 379(9822), 1225-1233.

Bashir, T., Macdonald, H.N. and Peacock, M., 1981. Biochemical evidence of vitamin D deficiency in pregnant Asian women. J Hum Nutr 35, 49-52.

Bassir, M., Laborie, S., Lapillonne, A., Claris, O., Chappuis, M.C. and Salle, B.L., 2001. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. Acta Paediatr 90, 577-579.

Brickley, M. and Ives, R., 2008. The bioarchaeology of metabolic bone disease. Academic Press, UK, pp 84.

Brooke, O.G., Brown, I.R., Bone, C.D., Carter, N.D., Cleeve. H.J., Maxwell, J.D., Robinson, V,P. and Winder, S.M., 1980. Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. Br Med J 280, 751-754.

Brooke, O.G., Brown, I.R., Cleeve, H.J. and Sood, A., 1981. Observations on thevitamin D state of pregnant Asian women in London. Br J Obstet Gynaecol 88, 18-26.

Brunvand, L., Shah, S.S., Bergström, S. and Haug, E., 1998. Vitamin D deficiency in pregnancy is not associated with obstructed labor. A study among Pakistani women in Karachi. Acta Obstet Gynecol Scand 77, 303-306.

Cheng, Y., Dibley, M.J., Zhang, X., Zeng, L. and Yan, H., 2009. Assessment of dietary intake among pregnant women in a rural area of western China. BMC Public Health 9, 222.

Cho, G.J., Shin, J.H., Yi, K.W., Park, H.T., Kim, T., Hur, J.Y. and Kim, S.H., 2012. Adolescent pregnancy is associated with osteoporosis in postmenopausal women. Menopause 19, 456-460.

Christian, P., Jiang, T., Khatry, S.K., LeClerq, S.C., Shrestha, S.R. and West Jr., K.P., 2006. Antenatal supplementation with micronutrients and biochemical indicators of status and subclinical infection in rural Nepal. Am J Clin Nutr 83, 788-794.

Clements, M.R., Johnson, L. and Fraser, D.R., 1987. A new mechanism for induced vitamin D deficiency in calcium deprivation. Nature 325, 62-65.

Datta, S., Alfaham, M., Davies, D.P., Dunstan, F., Woodhead, S., Evans, J. and Richards, B., 2002. Vitamin D deficiency in pregnant women from a non-European ethnic minority population – an interventional study. BJOG 109, 905-908.

De-Regil, L.M., Palacios, C., Ansary, A., Kulier, R. and Peña-Rosas, J.P., 2012. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2, CD008873.

Di Girolamo, L., Bond,T. C., Bramer, D., Diner, D. J., Fettinger, F., Kahn, R.A., Martonchik, J.V., Ramana, M.V., Ramanathan, V. and Rasch, P.J., 2004. Analysis of Multi-angle Imaging SpectroRadiometer (MISR) aerosol optical depths over greater India during winter 2001-2004. Geophysical Research Letters, 31, L23115.

Doi, M., Rekha, R.S., Ahmed, S., Okada, M., Roy, A.K., El Arifeen, S., Ekström, E.C., Raqib, R. and Wagatsuma, Y., 2011. Association between calcium in cord blood and newborn size in Bangladesh. Br J Nutr 106, 1398-1407.

Dror, D.K. and Allen, L.H., 2010. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. Nutr Rev 68, 465-477.

Du, X, Greenfield, H., Fraser, D.R., Ge, K., Trube, A. and Wang, Y., 2001. Vitamin D deficiency and associated factors in adolescent girls in Beijing. Am J Clin Nutr 74, 494-500.

Ergür, A.T., Berberoğlu, M., Atasay, B., Şıklar, Z., Bilir, P., Arsan, S., Söylemez, F. and Öcal, G., 2009. Vitamin D deficiency in Turkish mothers and their neonates and in women of reproductive age. J Clin Res Pediatr Endocrinol 1, 266-269.

Farrant, H.J., Krishnaveni, G.V., Hill, J.C., Boucher, B.J., Fisher, D.J., Noonan, K., Osmond, C., Veena, S.R. and Fall, C.H., 2009.Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. Eur J Clin Nutr 63, 646-652.

Feleke, Y., Abdulkadir, J., Mshana, R., Mekbib, T.A., Brunvand, L., Berg, J.P. and Falch, J.A., 1999. Low levels of serum calcidiol in an African population compared to a North European population. Eur J Endocrinol 141, 358-360.

Finkelstein, J.L., Mehta, S., Duggan, C., Manji, K.P., Mugusi, F.M., Aboud, S., Spiegelman, D., Msamanga, G.I. and Fawzi, W.W., 2012. Maternal vitamin D status and child morbidity, anemia, and growth in human immunodeficiency virus-exposed children in Tanzania. Pediatr Infect Dis J 31, 171-175.

Food and Agriculture Organization., 2001. Calcium. Available at: ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e00.pdf.

Gemens, T.L., Adams, J.S., Henderson, S.L. and Holick, M.F., 1982. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 319, 74-76.

Green, T.J., Skeaff, C.M., Rockell, J.E., Venn, B.J., Lambert, A., Todd, J., Khor, G.L., Loh, S.P., Muslimatun, S., Agustina, R. and Whiting. S.J., 2008. Vitamin D status and its association with parathyroid hormone concentrations in women of child-bearing age living in Jakarta and Kuala Lumpur. Eur J Clin Nutr 62, 373-378.

Halicioglu, O., Aksit, S., Koc, F., Akman, S.A., Albudak, E., Yaprak, I., Coker, I., Colak, A., Ozturk, C. and Gulec, E.S., 2012. Vitamin D deficiency in pregnant women and their neonates in spring time in western Turkey. Paediatr Perinat Epidemiol 26, 53-60.

Harinarayan, C.V., Ramalakshmi, T., Prasad, U.V., Sudhakar, D., Srinivasarao, P.V., Sarma, K.V. and Kumar, E.G., 2007. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. Am J Clin Nutr 85, 1062-1067.

Harvey, N.C., Javaid, K., Bishop, N., Kennedy, S., Papageorghiou, A.T., Fraser, R., Gandhi, S.V., Schoenmakers, I., Prentice, A. and Cooper. C., 2012. MAVIDOS Maternal Vitamin D Osteoporosis Study: study protocol for a randomized controlled trial. The MAVIDOS Study Group. Trials 13, 13.

Heere, C., Skeaff, C.M., Waqatakirewa, L., Vatucawaqa, P., Khan, A.N. and Green, T.J., 2010. Serum 25-hydroxyvitamin D concentration of Indigenous-Fijian and Fijian-Indian women. Asia Pac J Clin Nutr 19, 43-48.

Hodgkin, P., Hine, P.M., Kay, G.H., Lumb, G.A. and Stanbury, S.W., 1973. Vitamin-D deficiency in Asians at home and in Britain. Lancet 302(7822), 167-171.

Holick, M.F., MacLaughlin, J.A. and Doppelt, S.H., 1981. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science 211, 590-593.

Holick, M.F., 2007. Vitamin D deficiency. N Engl J Med 357, 266-281.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M., 2011. Evaluation, treatment, prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96, 1911-1930.

Hollis, B.W. and Wagner, C.L., 2004. Assessment of dietary vitamin D requirements during pregnancy and lactation. Am J Clin Nutr 79, 717-26.

Hollis, B.W. and Wagner CL., 2011. Vitamin D requirements and supplementation during pregnancy. Curr Opin Endocrinol Diabetes Obes 18, 371-375.

Hollis, B.W., Johnson, D., Hulsey, T.C., Ebeling, M. and Wagner CL., 2011. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res 26, 2341-2357.

Hossain, N., Khanani, R., Hussain-Kanani, F., Shah, T., Arif, S. and Pal, L., 2011. High prevalence of vitamin D deficiency in Pakistani mothers and their newborns. Int J Gynaecol Obstet 112, 229-233.

Ilahi, M., Armas, L.A. and Heaney, R.P., 2008. Pharmacokinetics of a single, large dose of cholecalciferol. Am J Clin Nutr 87, 688-691.

IOM (Institute of Medicine), 2011. Dietary reference intakes for calcium and vitamin D. Committee to Review Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press, Institute of Medicine, Washington, DC, USA.

Islam, M.Z., Akhtaruzzaman, M. and Lamberg-Allardt, C., 2006. Hypovitaminosis D is common in both veiled and nonveiled Bangladeshi women. Asia Pac J Clin Nutr 15, 81-87.

Islam, M.Z., Lamberg-Allardt, C., Kärkkäinen, M., Outila, T., Salamatullah, Q. and Shamim A.A., 2002. Vitamin D deficiency: a concern in premenopausal Bangladeshi women of two socio-economic groups in rural and urban region. Eur J Clin Nutr 56, 51-56.

Javaid, M.K., Crozier, S.R., Harvey, N.C., Gale, C.R., Dennison, E.M., Boucher, B.J., Arden, N.K., Godfrey, K.M., Cooper, C.and Princess Anne Hospital Study Group, 2006. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 367(9504), 36-43.

Jiang, L., Xu, J., Pan, S., Xie, E., Hu, Z. and Shen, H., 2012. High prevalence of hypovitaminosis D among pregnant women in southeast China. Acta Paediatr 101, e192-194.

Jiang, T., Christian, P., Khatry, S.K., Wu, L., West Jr., K.P., 2005. Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. J Nutr 135, 1106-1112.

Kabakyenga, J.K., Östergren, P.O., Turyakira, E., Mukasa, P.K. and Pettersson, K.O., 2011. Individual and health facility factors and the risk for obstructed labour and its adverse outcomes in south-western Uganda. BMC Pregnancy Childbirth 11, 73.

Kalra, P., Das, V., Agarwal, A., Kumar, M., Ramesh, V., Bhatia, E., Gupta, S., Singh, S., Saxena, P. and Bhatia, V., 2012. Effect of vitamin D supplementation during pregnancy on neonatal mineral homeostasis and anthropometry of the newborn and infant. Br J Nutr 108(6), 1052-1058.

Karim, S.A., Nusrat, U. and Aziz, S., 2011.Vitamin D deficiency in pregnant women and their newborns as seen at a tertiary-care center in Karachi, Pakistan. Int J Gynaecol Obstet 112, 59-62.

Konje, J.C. and Ladipo, O.A., 2000. Nutrition and obstructed labor.Am J Clin Nutr 72, 291S-297S.

Leffelaar, E.R., Vrijkotte, T.G. and van Eijsden. M., 2010. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. Br J Nutr 104, 108-117.

Lips, P., 2007. Vitamin D status and nutrition in Europe and Asia. J. Steroid Biochem. Mol. Biol. 103, 620-625.

Lo, C.W., Paris, P.W. and Holick, M.F., 1986. Indian and Pakistani immigrants have the same capacity as Caucasians to produce vitamin D in response to ultraviolet irradiation. Am J Clin Nutr 44, 683-685.

Madar, A.A., Stene, L.C. and Meyer, H.E., 2009. Vitamin D status among immigrant mothers from Pakistan, Turkey and Somalia and their infants attending child health clinics in Norway. Br J Nutr 101, 1052-1058.

Madelenat, P., Bastian, H. and Menn, S., 2001. Winter supplementation in the 3rd trimester of pregnancy by a dose of 80,000 IU of vitamin D. J Gynecol Obstet Biol Reprod (Paris) 30, 761-767.

Maghbooli, Z., Hossein-Nezhad, A,, Shafaei, A.R., Karimi, F., Madani, F.S. and Larijani, B., 2007. Vitamin D status in mothers and their newborns in Iran. BMC Pregnancy Childbirth 12, 1.

Marwaha, R.K., Tandon, N., Chopra, S., Agarwal, N., Garg, M.K., Sharma, B., Kanwar, R.S., Bhadra, K., Singh, S., Mani, K. and Puri, S., 2011. Vitamin D status in pregnant Indian women across trimesters and different seasons and its correlation with neonatal serum 25-hydroxyvitamin D levels. Br J Nutr 106, 1383-1389.

Marya, R.K., Rathee, S., Dua, V. and Sangwan, K., 1988. Effect of vitamin D supplementation during pregnancy on foetal growth. Indian J Med Res 88, 488-492.

Matsuoka, L.Y, Wortsman, J., Haddad, J.G., Kolm, P. and Hollis, B.W., 1991. Racial pigmentation and the cutaneous synthesis of vitamin D. Arch Dermatol 127, 536-538.

Maxwell, J.P. and Miles, L.M., 1925. Osteomalacia in China. Proc R Soc Med 18 (Obstet Gynaecol Sect), 48-66.

McIntire, D.D., Bloom, S.L., Casey, B.M. and Leveno, K.J., 1999. Birth weight in relation to morbidity and mortality among newborn infants. N Engl J Med 340, 1234-1238.

Mehta, S., Hunter, D.J., Mugusi, F.M., Spiegelman, D., Manji, K.P., Giovannucci, E.L., Hertzmark, E., Msamanga, G.I. and Fawzi, W.W., 2009. Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania. J Infect Dis 200, 1022-1030.

Mehta, S., Spiegelman, D., Aboud, S., Giovannucci, E.L., Msamanga, G.I., Hertzmark, E., Mugusi, F.M., Hunter, D.J. and Fawzi, W.W., 2010. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. Eur J Clin Nutr 64, 808-817.

Merewood, A., Mehta, S.D., Chen, T.C., Bauchner, H. and Holick, M.F., 2009. Association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab 94, 940-945.

Mozaffari-Khosravi, H., Hosseinzadeh-Shamsi-Anar, M., Salami, M.A., Hadinedoushan, H. and Mozayan, M.R., 2012. Effects of a single post-partum injection of a high dose of vitamin D on glucose tolerance and insulin resistance in mothers with first-time gestational diabetes mellitus. Diabet Med 29, 36-42.

Namgung, R., Tsang, R.C., Lee, C., Han, D.G., Ho, M.L. and Sierra, R.I., 1998. Low total body bone mineral content and high bone resorption in Korean winter-born versus summer-born newborn infants. J Pediatr 132, 421-425.

Oliveri, M.B., Mautalen, C.A., Alonso, A., Velázquez, H., Trouchot, H.A., Porto, R., Martinez, L. and Barata, A.D., 1993. Nutritional status of vitamin D in mothers and neonates of Ushuaia and Buenos Aires. Medicina (B Aires) 53, 315-20.

Pallikadavath, S., Foss, M. and Stones, R.W., 2004. Antenatal care: provision and inequality in rural north India. Soc Sci Med 59, 1147-58.

Panwar, B. and Punia, D., 2000. Analysis of composite diets of rural pregnant women and comparison with calculated values. Nutr Health 14, 217-23.

Population Reference Bureau, 2011. Who Speaks for Me? Ending Child Marriage. Washington, DC, USA. Available at: http://www.prb.org/pdf11/ending-child-marriage.pdf.

Prentice, A., Jarjou, L.M., Goldberg, G.R., Bennett, J., Cole, T.J. and Schoenmakers, I., 2009a. Maternal plasma 25-hydroxyvitamin D concentration and birthweight, growth and bone mineral accretion of Gambian infants. Acta Paediatr 98, 1360-1362.

Prentice, A., Schoenmakers, I., Jones, K., Jarjou, L. and Goldberg, G., 2009b. Vitamin D Deficiency and Its Health Consequences in Africa. Clinical Review in Bone and Mineral Metabolism 7, 94-106.

Prentice, A., 2011. Milk intake, calcium and vitamin D in pregnancy and lactation: effects on maternal, fetal and infant bone in low- and high-income countries. Nestle Nutr Workshop Ser Pediatr Program 67, 1-15.

Ramanathan, V., Chung, C., Kim, D., Bettge, T., Buja, L., Kiehl, J.T., Washington, W.M., Fu, Q., Sikka, D.R. and Wild. M., 2005. Atmospheric brown clouds: impacts on South Asian climate and hydrological cycle. Proc Natl Acad Sci U S A, 102, 5326-5333.

Rosen, C.J., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Manson, J.E., Mayne, S.T., Ross, A.C., Shapses, S.A. and Taylor, C.L., 2012. IOM Committee Members Respond to Endocrine Society Vitamin D Guideline. J Clin Endocrinol Metab 97, 1146-1152.

Roth, D.E., 2011. Vitamin D supplementation during pregnancy: safety considerations in the design and interpretation of clinical trials. Journal of Perinatology 31, 449-459.

Saadi, H.F., Dawodu, A., Afandi, B.O., Zayed, R., Benedict, S. and Nagelkerke, N., 2007. Efficacy of daily and monthly high-dose calciferol in vitamin D-deficient nulliparous and lactating women. Am J Clin Nutr 85, 1565-1571.

Sachan, A., Gupta, R., Das, V., Agarwal, A., Awasthi, P.K. and Bhatia, V., 2005. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. Am J Clin Nutr 81, 1060-1064.

Sahu, M., Bhatia, V., Aggarwal, A., Rawat, V., Saxena, P., Pandey, A. and Das, V., 2009a. Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India. Clin Endocrinol (Oxf) 70, 680-684.

Sahu, M., Das, V., Aggarwal, A., Rawat, V., Saxena, P. and Bhatia, V., 2009b. Vitamin D replacement in pregnant women in rural north India: a pilot study. Eur J Clin Nutr 63, 1157-1159.

Specker, B.L., 2012. Does vitamin D during pregnancy impact offspring growth and bone? Proc Nutr Soc 71, 38-45.

Thandrayen, K., and Pettifor, J.M., 2010. Maternal vitamin D status: implications for the development of infantile nutritional rickets. Endocrinol Metab Clin North Am 39, 303-320.

Van der Meer, I.M., Karamali, N.S., Boeke, A.J., Lips, P., Middelkoop, B.J., Verhoeven, I. and Wuister, J.D., 2006. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. Am J Clin Nutr 84, 350-353.

Van Schoor, N.M. and Lips, P., 2011. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab 25, 671-680.

Vieth R., 2011. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/l (30 ng/ml). Best Pract Res Clin Endocrinol Metab 25, 681-691.

Wang, J., Yang, F., Mao, M., Liu, D.H., Yang, H.M. and Yang, S.F., 2010. High prevalence of vitamin D and calcium deficiency among pregnant women and their newborns in Chengdu, China. World J Pediatr 6, 265-267.

Watney, P.J., Chance, G.W., Scott, P. and Thompson, J.M., 1971. Maternal factors in neonatal hypocalcaemia: a study in three ethnic groups. Br Med J 2(5759), 432-436.

World Bank, 2012. How we Classify Countries. Available at: http://data.worldbank.org/about/country-classifications.

World Health Organization (WHO), 2009. Adult women: The reproductive years. In: Women and health: today's evidence tomorrow's agenda. WHO Press, Geneva, Switzerland, pp 39.

Yu, C.K., Sykes, L., Sethi, M., Teoh, T.G. and Robinson, S., 2009. Vitamin D deficiency and supplementation during pregnancy. Clin Endocrinol (Oxf) 70, 685-690.

Key facts

- Vitamin D metabolism adapts to the physiological state of pregnancy.
- Vitamin D inadequacy is highly prevalent during pregnancy despite the inclusion of vitamin D in prenatal vitamin supplements in many countries.
- Maternal vitamin D inadequacy may impact multiple maternal, fetal, and postnatal outcomes.
- There is no consensus on optimal vitamin D status during pregnancy or the intake necessary to achieve it.
- No adverse outcomes have been demonstrated in trials supplementing pregnant women with vitamin D doses up to 100 μg/d.

Summary points

- 25-hydroxyvitamin D is the predominant form of vitamin D to cross the placenta.
- Fetal vitamin D status is dependent on maternal status.
- Vitamin D inadequacy is highly prevalent among pregnant populations worldwide.
- While there is no consensus on defining optimal vitamin D status during pregnancy, inadequacy may adversely affect pregnancy outcomes and offspring health.
- There is inconsistent evidence that vitamin D inadequacy before and during pregnancy may predispose women for infertility, preeclampsia, gestational diabetes, bacterial vaginosis, spontaneous preterm birth, Cesarean section, periodontal disease, and human immunodeficiency virus progression and transmission.
- Gestational vitamin D deficiency may impact fetal skeletal development, intrauterine growth, immune maturation, and brain development.
- Poor vitamin D status *in utero* could place offspring at higher risk for development of asthma, type I diabetes, schizophrenia, and multiple sclerosis.
- Current vitamin D intake recommendations during pregnancy are 5-15 μg/d, though recent evidence suggests that higher intakes are necessary to achieve and maintain vitamin D adequacy.
- Supplementation with 25-100 μg/d vitamin D during pregnancy has not resulted in adverse pregnancy or birth outcomes.

34. Vitamin D in pregnancy

D. Dror
Western Human Nutrition Research Center, 430 W. Health Sciences Dr., Davis, CA 95616, USA;
dkdror@ucdavis.edu

Abstract

Pregnancy is a time of dynamic change in mother and fetus, with vitamin D playing a key role in the normal development and performance of multiple physiological systems. Maternal vitamin D metabolism is modified during pregnancy as evidenced by elevated circulating concentrations of vitamin D binding protein and 1,25-dihydroxyvitamin D. Maternal 25-hydroxyvitamin D (25(OH)D), which shows little change in pregnancy, is the main vitamin D metabolite to cross the placenta. Although there is no consensus on optimal 25(OH)D concentrations during pregnancy, suboptimal status is prevalent in populations of pregnant women around the world. Impairment of vitamin D status during pregnancy has been investigated with regards to multiple maternal outcomes including infertility, preeclampsia, gestational diabetes, bacterial vaginosis, spontaneous preterm birth, mode of delivery, periodontal disease, and human immunodeficiency virus progression and vertical transmission, though evidence for the majority of these outcomes is inconsistent. Gestational vitamin D status may influence skeletal development, intrauterine growth, immune maturation, and neural development of the fetus. Vitamin D inadequacy *in utero* may program for later life health outcomes, potentially increasing the risk of asthma, type I diabetes, schizophrenia, and multiple sclerosis in the offspring. Vitamin D intake recommendations during pregnancy in most countries range from 5-15 μg/d. However, in the absence of sun exposure such intakes may be insufficient to achieve adequate circulating 25(OH)D, and several expert committees have recommended vitamin D intakes from 25-100 μg/d during pregnancy. While adverse effects have not been reported in trials utilizing these doses, additional research is necessary to verify long-term safety to mother and offspring and to establish a clinical protocol for monitoring and maintaining vitamin D adequacy during pregnancy.

Keywords: mother, fetus, deficiency, 25(OH)D

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_34, © Wageningen Academic Publishers 2013

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
BMC	Bone mineral content
BV	Bacterial vaginosis
CI	Confidence interval
CSA	Cross-sectional area
DBP	Vitamin D binding protein
GDM	Gestational diabetes mellitus
HbA_{1c}	Glycated hemoglobin
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ILT	Immunoglobulin-like transcripts
MS	Multiple sclerosis
OR	Odds ratio
RCT	Randomized controlled trial
RR	Relative risk
SPB	Spontaneous preterm birth
SGA	Small for gestational age
Th1	T helper cell type 1
Th2	T helper cell type 2
VDD	Vitamin D deficiency
VDR	Vitamin D receptor

34.1 Introduction

Despite increasing awareness of the multiple roles of vitamin D in pregnancy and fetal development, vitamin D inadequacy is prevalent during pregnancy in diverse populations around the world. While optimal vitamin D status during pregnancy and intake requirements to achieve it are widely debated, there is general consensus that inadequate maternal 25(OH)D concentration can adversely affect mother and offspring. This chapter discusses adaptations of vitamin D metabolism during pregnancy, evidence of the potential consequences of inadequacy, and considerations regarding vitamin D intake and supplementation.

34.2 Adaptations of vitamin D metabolism during pregnancy

The multifold physiological changes of pregnancy include an increase in circulating concentrations of DBP and $1,25(OH)_2D$ with little change in 25(OH)D, the main biomarker of vitamin D status (Papapetrou, 2010). DBP, the major binding protein for 25(OH)D and $1,25(OH)_2D$, is abundant in circulation and binds 85% to 90% of both forms of vitamin D while albumin binds most of

the remaining 10-15% (Bikle *et al.*, 1985, 1986). DBP increases in pregnancy, with an estimated elevation of 7% to 152% during the course of pregnancy beginning as early as 8-10 weeks gestation (Brannon and Picciano, 2011; Ritchie *et al.*, 1998). The pregnancy-induced rise in DBP may be related to estrogen levels, supported by evidence of higher serum levels of DBP in women taking estrogen-containing contraceptives (Haddad and Walgate, 1976; Van Hoof *et al.*, 2001) or treated with oral estrogen (Bikle *et al.*, 1992; Dick *et al.*, 1995).

Total serum $1,25(OH)_2D$ is elevated from early pregnancy and rises steadily to a peak concentration nearly double that of non-pregnant controls by term (Kovacs, 2008). The rise in circulating DBP during pregnancy accounts in part for elevated total $1,25(OH)_2D$, with which it is significantly correlated (Bikle *et al.*, 1984; Bouillon *et al.*, 1981). Biologically active free serum $1,25(OH)_2D$ is comparable to pre-pregnant concentrations except in the final trimester, when investigators have found it variably to be normal or elevated (Bikle *et al.*, 1984; Bouillon *et al.*, 1981; Van Hoof *et al.*, 2001). The fact that placental and decidual mRNA expression of 1-α-hydroxylase, the enzyme required to synthesize $1,25(OH)_2D$ from 25(OH)D, increases early in pregnancy while $1,25(OH)_2D$ peaks late in pregnancy suggests maternal rather than placental origin (Zehnder *et al.*, 2002). However, it has recently been demonstrated *in vitro* that methylation of the placental *CYP24A1* gene promoter directly down-regulates 24-hydroxylase mediated catabolism of $1,25(OH)_2D$ (Novakovic *et al.*, 2009), possibly contributing to increased $1,25(OH)_2D$ concentrations observed during pregnancy. Cord blood $1,25(OH)_2D$ is not elevated during gestation, suggesting different physiological use between mother and fetus (Liu and Hewison, 2012).

The correlation between maternal and cord 25(OH)D, but not $1,25(OH)_2D$, suggests that 25(OH)D is the predominant vitamin D metabolite to cross the placenta and that fetal status is dependent on maternal status (Brannon and Picciano, 2011). Concentrations of 25(OH)D do not increase significantly in pregnant women compared with non-pregnant controls (Papapetrou, 2010). In fact, early increases in decidual and placental 1-α-hydroxylase activity may lower maternal 25(OH)D concentrations (Lanham-New *et al.*, 2011). While elevated concentrations of $1,25(OH)_2D$ increase catabolism of 25(OH)D in non-pregnant rats and humans, this regulation appears to be uncoupled during pregnancy as evidenced by a ratio of $1,25(OH)_2D$/1,25(OH)D 2.5 times higher than in the non-pregnant state (Papapetrou, 2010).

34.3 Prevalence of vitamin D inadequacy during pregnancy

Though circulating 25(OH)D is considered the best biomarker of dietary exposure and endogenous synthesis, there is no consensus on definitions of status. The most recent United States Institute of Medicine recommendations, released in 2010, consider maternal 25(OH)D concentrations equal to or above 50 nmol/l during pregnancy to meet the needs of 97.5% of the population (Ross *et al.*, 2010). However, 25(OH)D concentrations above 75-80 nmol/l are considered sufficient based on associations with PTH concentrations, calcium absorption, bone turnover markers, and bone mineral density (Dawson-Hughes *et al.*, 2005; Holick, 2007; Hollis,

2005). The majority of recent prevalence estimates of vitamin D inadequacy during pregnancy have used serum 25(OH)D concentration below 50 nmol/l as a cut-off for deficiency and below 75-80 nmol/l as a cut-off for insufficiency. In the United States, Canada, Australia, Europe, the Middle East, and South Asia 26-98% of pregnant women at or near term are deficient and 66-100% are insufficient in vitamin D, with a trend of higher levels of deficiency and insufficiency in dark-skinned or shrouded women (Dror, 2011; Liu and Hewison, 2012).

34.4 Vitamin D and pregnancy outcomes

A number of maternal, fetal, and postnatal outcomes have been correlated with poor vitamin D status during pregnancy. Maternal pregnancy characteristics investigated in association with compromised vitamin D status include infertility, preeclampsia, GDM, mode of delivery, BV, periodontal disease, and HIV progression and vertical transmission, though evidence for many of these outcomes is inconclusive. In infants, attention has been devoted to the impact of the *in utero* vitamin D environment on bone mineralization and skeletal development, intrauterine growth, immune maturation, and brain development. There is some evidence suggesting long-term consequences of compromised vitamin D status during gestation, with increased offspring risk for later development of asthma, type I diabetes, schizophrenia, and MS.

34.5 Maternal outcomes

34.5.1 Infertility

The role of vitamin D in maternal fertility is poorly understood, but experimental and ecological evidence suggest that vitamin D may be involved in successful implantation and maintenance of pregnancy. In VDD, VDR-ablated, or 1-α-hydroxylase null animal models, adult females had reduced fertility and smaller litter sizes (Kovacs, 2008). In humans, the VDR is expressed in the ovaries, pituitary gland, endometrium, and placenta (Lerchbaum and Obermayer-Pietsch, 2012) and conception rates peak in summer in northern countries with strong seasonal contrasts in luminosity (Rojansky *et al.*, 1992). It has been speculated that the rise in $1,25(OH)_2D$ concentrations early in pregnancy is necessary for ensuring immunological adaptations, including dampening Th1 function and favoring Th2 domination for immune tolerance of implantation and pregnancy maintenance (Evans *et al.*, 2004; Hypponen, 2011). However, studies measuring serum and follicular fluid concentrations of 25(OH)D and achievement of clinical *in vitro* fertilization are inconclusive, variably finding positive (Ozkan *et al.*, 2010), negative (Anifandis *et al.*, 2010), and null (Aleyasin *et al.*, 2011) associations between vitamin D status and reproductive success.

34.5.2 Preeclampsia

Preeclampsia, defined as hypertension and proteinuria after 20 weeks gestation, occurs in 3-5% of pregnancies worldwide and is the leading cause of maternal and fetal morbidity and mortality

(Roberts and Lain, 2002). Higher incidence of preeclampsia during winter in non-tropical regions and during wet or humid periods in tropical climates (TePoel *et al.*, 2011) as well as a higher incidence in dark-skinned compared with light-skinned women (Mostello *et al.*, 2002) suggest a possible influence of sunlight and vitamin D status. Furthermore, it has been postulated that the adverse maternal response to the fetus that occurs in preeclampsia is mediated by Th1-type cytokines (Saito and Sakai, 2003), which are favored in vitamin D deficiency. There is consistent evidence of $1,25(OH)_2D$ suppression in preeclamptic compared with normal pregnancies (August *et al.*, 1992; Halhali *et al.*, 1995, 2000, 2004; Seely *et al.*, 1992), supporting a disruption in immune tolerance mediated by altered vitamin D metabolism.

Several case-control studies have found significantly lower maternal 25(OH)D concentration in the second or third trimester of pregnancy in women who subsequently developed preeclampsia (Baker *et al.*, 2010; Bodnar *et al.*, 2007; Robinson *et al.*, 2010). In a nested-case control study, maternal 25(OH)D concentration below 50 nmol/l at 15-20 weeks gestation was associated with a five-fold increase in the adjusted odds of severe preeclampsia diagnosis (adjusted OR 5.41, 95% CI 2.02 to 14.52) (Baker *et al.*, 2010). In contrast, other studies found no association between first or second trimester 25(OH)D and later development of preeclampsia in women at low- or high risk based on clinical or biochemical factors (Powe *et al.*, 2010; Shand *et al.*, 2010; Yu *et al.*, 2012). A single RCT in India involving supplementation of pregnant women with 30 μg vitamin D and 375 mg calcium daily starting at 20-24 weeks gestation found no difference in incidence of preeclampsia between the treatment and control groups (Marya *et al.*, 1987).

34.5.3 Gestational diabetes

GDM is increasingly prevalent and has long-term health implications for both mother and offspring (Grundmann and von Versen-Hoynck, 2011). It has been postulated that 25(OH)D and $1,25(OH)_2D$ may directly or indirectly modulate pancreatic β-cell function and insulin sensitivity (McLeod *et al.*, 2012; Takiishi *et al.*, 2010), but confounders such as genetic polymorphisms and obesity can predispose women both for poor vitamin D status and GDM (Begum *et al.*, 2011; Ramos-Lopez *et al.*, 2008). Some studies have found significantly lower maternal 25(OH)D concentrations in women with GDM compared with pregnant controls (Lau *et al.*, 2011; Maghbooli *et al.*, 2008; Parlea *et al.*, 2012; Soheilykhah *et al.*, 2010; Zhang *et al.*, 2008), while others have found no association between vitamin D status during pregnancy and GDM (Baker *et al.*, 2012; Farrant *et al.*, 2009). Plasma 25(OH)D concentration at 16 weeks gestation was significantly lower in women who subsequently developed GDM compared with matched controls following adjustment for multiple confounders in a nested case-control study, with a 12.5 nmol/l decrease in 25(OH)D associated with a 30% increase in odds of GDM (adjusted OR 1.29, 95% CI 1.05 to 1.60) (Zhang *et al.*, 2008). In another study, 25(OH)D at mid-gestation was negatively correlated with fasting glucose, fasting insulin, and insulin resistance, but the odds of developing GDM was not significantly higher in women who had 25(OH)D concentrations below 50 nmol/l at mid-gestation compared to those with better vitamin D status (OR 1.92, 95% CI 0.89 to 4.17) (Clifton-Bligh *et al.*, 2008). In the first study to measure vitamin D status and HbA_{1c}, an indicator of long-term blood glucose control, in women with GDM, third trimester 25(OH)D

was significantly inversely associated with HbA_{1c} ($P<0.001$) and was an independent predictor of HbA_{1c} in multivariate modeling, though no association was found between low vitamin D status and diagnosis of GDM (Lau *et al.*, 2011).

34.5.4 Bacterial vaginosis

BV, a prevalent vaginal infection associated with adverse pregnancy outcomes including preterm delivery, is consistently inversely associated with vitamin D status during pregnancy. In early gestation, mean serum 25(OH)D concentration was significantly lower in women with BV compared with controls with normal vaginal flora ($P<0.001$) (Bodnar *et al.*, 2009). Later studies have corroborated an inverse association between 25(OH)D and BV in pregnant women and adolescents (Davis *et al.*, 2010; Dunlop *et al.*, 2011; Hensel *et al.*, 2011), with a higher prevalence of BV in dark-skinned than Caucasian pregnant women supporting potential involvement of vitamin D.

34.5.5 Length of gestation/spontaneous preterm birth

SPB occurs prior to 37 weeks gestation, with numerous pathophysiological causes including intrauterine infections and inflammation. It has been postulated that the immunomodulatory and anti-inflammatory properties of vitamin D may be protective against SPB (Grundmann and von Versen-Hoynck, 2011). In a single observational study, length of gestation was 0.7 weeks shorter in a small subset of women (7%) with 25(OH)D below 28 nmol/l at 28-32 weeks gestation (95% CI -1.3 to -0.1) (Morley *et al.*, 2006). However, vitamin D status or supplementation was not associated with length of gestation or risk of SPB in later studies (Baker *et al.*, 2011; Dror *et al.*, 2011; Hollis *et al.*, 2011; Mehta *et al.*, 2009; Shand *et al.*, 2010; Yu *et al.*, 2009).

34.5.6 Mode of delivery

Historically, severe vitamin D deficiency and rickets were known to cause pelvic deformities, increasing the risk of obstructed labor (Urrutia and Thorp, 2012). In a study conducted in the United States, women with 25(OH)D concentrations below 37.5 nmol/l following delivery were nearly four times more likely to have required a Cesarean section than women with better vitamin D status (adjusted OR 3.84, 95% CI 1.71 to 8.62) (Merewood *et al.*, 2009). However, other studies in Australia, Pakistan, the United States and the United Kingdom have failed to corroborate this finding (Bowyer *et al.*, 2009; Brunvand *et al.*, 1998; Dror *et al.*, 2011; Savvidou *et al.*, 2012).

34.5.7 Other

Several other conditions have been investigated with regards to vitamin D status during pregnancy, including periodontal disease and HIV progression and vertical transmission. Serum 25(OH)D at 14-26 weeks gestation was significantly lower in pregnant women with clinically moderate to severe periodontal disease compared with periodontally healthy controls ($P<0.001$) (Boggess *et al.*, 2011). A longitudinal study following 884 HIV-infected Tanzanian women for approximately

6 years found that women in the highest quintile of serum 25(OH)D at 12-27 weeks gestation had a 42% lower risk of all-cause mortality compared with women in the lowest quintile (relative risk 0.58, 95% CI 0.40 to 0.84) (Mehta *et al.*, 2010). Serum 25(OH)D below 80 nmol/l at mid-gestation was associated with greater maternal HIV disease progression (RR 1.25, 95% CI 1.05 to 1.50) and a higher risk of maternal to child transmission by 6 weeks (RR 1.50, 95% CI 1.02 to 2.20) and 24 months (RR 1.46, 95% CI 1.11 to 1.91) postpartum (Mehta *et al.*, 2009, 2010).

34.6 Fetal and neonatal outcomes

34.6.1 Skeletal development

Despite a recognized association between poor maternal vitamin D status and early development of infantile rickets, the role of vitamin D in fetal skeletal development and bone mineralization is uncertain. In South Korea, where vitamin D supplementation is uncommon, investigators reported significantly lower mean cord serum 25(OH)D concentrations and total body BMC in winter-born compared with summer-born neonates (Namgung *et al.*, 1998). In a prospective cohort study using high-resolution three-dimensional ultrasound imaging, maternal serum 25(OH)D was inversely correlated with fetal femoral distal metaphyseal CSA (r=-0.16, 95% CI -0.25 to -0.06) and splaying index (metaphyseal CSA/femur length, r=-0.17, 95% CI -0.26 to -0.07) at 19 weeks gestation, with similar results at 34 weeks gestation. Fetal femoral splaying is analogous to that seen in childhood rickets, suggesting that the effects of vitamin D deficiency on bone development may initiate early in gestation (Mahon *et al.*, 2010). Tibial BMC and CSA measured by peripheral quantitative computed tomography were significantly higher in Finnish newborns whose mothers' serum 25(OH)D in the first trimester of pregnancy was above median (46.2 nmol/l, P=0.01 and P=0.02) (Viljakainen *et al.*, 2010). By 14 months, tibial CSA remained significantly higher in the group of mothers with better vitamin D status during pregnancy (P=0.004), but there was no residual difference in BMC between groups (Viljakainen *et al.*, 2011). In contrast, studies in Turkey, The Gambia, and the United Kingdom found no significant association between maternal or cord 25(OH)D and unadjusted neonatal BMC or bone area, though the time of measurement, range of vitamin D status, and methods of assessing bone parameters differed between studies (Akcakus *et al.*, 2006; Congdon *et al.*, 1983; Prentice *et al.*, 2009; Weiler *et al.*, 2005).

34.6.2 Intrauterine growth

While fetal growth is maximal in the third trimester, the growth trajectory is determined earlier in pregnancy by factors possibly including vitamin D (Bodnar *et al.*, 2010). A large multiethnic cohort study in the Netherlands reported that compared with infants of mothers whose serum 25(OH)D at 12-14 weeks gestation was greater than 50 nmol/l, those born to mothers with serum 25(OH)D below 29.9 nmol/l had significantly lower birth weights (-114.4 g, 95% CI=-151.2 to -77.6) and were more likely to be SGA (OR 2.4, 95% CI 1.9 to 3.2) (Leffelaar *et al.*, 2010). Women in the lowest quartile of serum 25(OH)D during early pregnancy were more likely to give birth

to SGA offspring after adjustment for maternal age, height, and education in another Dutch population-based study (adjusted OR 1.57, 95% CI 1.03 to 2.39) (Van den Berg *et al.*, 2012). Knee-heel, but not crown-heel, length was significantly shorter at birth in infants of a small subset of mothers (7%) with serum 25(OH)D below 28 nmol/l at 28-32 weeks gestation after adjustment for gestational length (-2.7 mm, 95% CI -5.4 to -0.1) and in winter-born vs. summer-born infants (-2.1 mm, 95% CI -3.8 to -0.5) (Morley *et al.*, 2006). In contrast, a number of observational studies have found no association between third trimester or perinatal maternal vitamin D status and birth weight, length, or head circumference (Akcakus *et al.*, 2006; Bowyer *et al.*, 2009; Dror *et al.*, 2011; Farrant *et al.*, 2009; Gale *et al.*, 2008; Prentice *et al.*, 2009; Yu *et al.*, 2009), while one found an inverse association between maternal 25(OH)D concentration at term and birth weight (Weiler *et al.*, 2005).

A nested case-control study of nulliparous pregnant women in the United States demonstrated a significant association between early pregnancy serum 25(OH)D and risk of SGA amongst white but not black women. White mothers with serum 25(OH)D below 37.5 nmol/l had a significantly greater odds of an SGA infant than those with serum 25(OH)D 37.5-75 nmol/l (OR 7.5, 95% CI 1.8 to 31.9) (Bodnar *et al.*, 2010). Similar results were found in the United Kingdom, where there was a significantly higher incidence of maternal serum 25(OH)D below the 10th percentile at 11-13 weeks gestation in Caucasian women (P=0.0002) but not in African women who delivered SGA neonates compared with those whose neonates were appropriate for gestational age (Ertl *et al.*, 2012). Single nucleotide polymorphisms in the VDR gene may contribute to disparities in fetal growth (Bodnar *et al.*, 2010; Morley *et al.*, 2009; Swamy *et al.*, 2011).

A recent Cochrane analysis including five RCTs found no effect of supplementation with 20-30 μg/d vitamin D during the third trimester of pregnancy on birth weight or birth length (De-Regil *et al.*, 2012). Likelihood of low birth weight (<2,500 g) was lower in the supplemented groups with borderline significance (RR 0.48, 95% CI 0.23 to 1.01), and head circumference was greater in the supplemented groups (mean difference 0.43 cm, 95% CI 0.06 to 0.79) (De-Regil *et al.*, 2012). In an RCT in India published after the Cochrane review, either a single oral dose of 1,500 μg vitamin D during the second trimester or two doses of 3,000 μg during the second trimester and at 28 weeks gestation resulted in significantly greater birth weight, length and head circumference compared with unsupplemented controls (P<0.01 for weight, P<0.001 for length and head circumference), with significant differences in anthropometric indices remaining between treatment and control groups at 3, 6, and 9 months (Kalra *et al.*, 2012).

34.6.3 Immune maturation

The gestational vitamin D environment may influence fetal immune development and later propensity for allergy. A multicenter trial in Finland and France found significantly higher expression of tolerogenic ILT3 and ILT4 in cord blood of infants born to mothers taking vitamin D supplements during pregnancy (P=0.012 and P<0.001) (Rochat *et al.*, 2010). A weak but significant positive correlation between cord plasma 25(OH)D and cord blood mononuclear cell release of interferon-γ, a cytokine playing a key role in Th1 cell development, was shown upon

stimulation with lipopolysaccharide (r=0.11, *P*=0.01) (Chi *et al.*, 2011). This finding suggests that prenatal vitamin D status could influence immune development and predisposition for allergy, though it must be interpreted cautiously given multiple correlation tests performed. Inverse associations between cord 25(OH)D and respiratory and general infections in neonates have been found in several recent studies (Belderbos *et al.*, 2011; Camargo *et al.*, 2011; Morales *et al.*, 2012).

34.6.4 Brain development

Vitamin D directly regulates development of neuronal and non-neuronal cells, with disruption of these processes potentially impairing cell viability, cell signaling or connectivity (Eyles *et al.*, 2011). A number of studies on gestational VDD and brain development have been conducted in rats, which like humans, express 1-α-hydroxylase and nuclear VDR in the brain (Levenson and Figueiroa, 2008). The brain morphology of rat pups born to VDD dams is altered, consistent with enhanced cell proliferation and decreased neuronal differentiation and apoptosis (Cui *et al.*, 2007; Eyles *et al.*, 2003; Ko *et al.*, 2004). Although similar results are not available from humans, there is ample evidence for a role of vitamin D in brain development and function (McCann and Ames, 2008). A recent study evaluating cognitive development longitudinally in children whose mothers' serum 25(OH)D was measured at 18 weeks gestation found a significant inverse trend between quartiles of maternal vitamin D status and language impairment at age 5 and 10 years ($P<0.05$), but not between vitamin D status and behavioral or emotional problems at any age (Whitehouse *et al.*, 2012).

34.7 Developmental programming for later life outcomes

The 'fetal origins' hypothesis that prenatal environmental factors influence disease risk in childhood and adulthood has stimulated speculation about the long-term implications of vitamin D status during gestation (Barker *et al.*, 2002; Lucas *et al.*, 2008). Epigenetic mechanisms that lead to persistent changes in structure and function of the endocrine system are hypothesized to account for lasting impacts of the intrauterine environment (Grundmann and von Versen-Hoynck, 2011).

34.7.1 Childhood asthma and wheezing

Vitamin D is a factor in numerous biological pathways affected by asthma, including smooth muscle contraction, airway inflammation, and immune cell function (Litonjua and Weiss, 2007). Several prospective birth cohort studies demonstrated that higher maternal intake of vitamin D from foods and supplements during pregnancy was associated with lower risk of wheezing (Camargo *et al.*, 2007; Devereux *et al.*, 2007; Miyake *et al.*, 2010) or asthma (Erkkola *et al.*, 2009) in children aged 16 months to five years. Only two prospective studies have measured maternal or cord 25(OH)D in association with wheezing and asthma. In a New Zealand birth cohort, cord blood 25(OH)D was inversely associated with risk of wheezing by 15 months, three years, and five years of age after adjustment for confounders ($P<0.05$ for all) but was not associated with

incident asthma by 5 years (Camargo *et al.*, 2011). In Spain, no association was found between early pregnancy 25(OH)D and risk of wheezing or asthma in offspring up to six years of age (Morales *et al.*, 2012).

34.7.2 Type I diabetes

While the destruction of insulin-secreting pancreatic beta cells characterizing type I diabetes may be initiated before birth (Deluca and Cantorna, 2001), evidence of an association between maternal vitamin D intake from food and supplements or maternal vitamin D status during pregnancy and risk of type I diabetes in the offspring is inconsistent. In a United States birth cohort followed for an average of four years, recalled maternal intake of vitamin D from food but not supplements during the third trimester of pregnancy was associated with a lower risk of pancreatic islet autoimmunity, a preclinical stage of type I diabetes, after adjustment for HLA genotype, family history of type 1 diabetes, presence of GDM, and ethnicity (adjusted hazard ratio 0.37, 95% CI 0.17 to 0.78; vitamin D intake standard deviation (SD) = 155.6 IU) (Fronczak *et al.*, 2003). In Sweden, daily consumption of prenatal supplements containing at least 5 μg vitamin D during pregnancy was associated with reduced diabetes-related autoimmunity in offspring at age one (adjusted OR 0.71, 95% CI 0.52 to 0.96) but not two and a half years (Brekke and Ludvigsson, 2007). However, in a Finnish cohort of children with a high-risk HLA genotype for type I diabetes, maternal vitamin D intake from food and/or supplements during pregnancy was not associated with risk of advanced beta cell autoimmunity or clinical type I diabetes in the offspring (Marjamaki *et al.*, 2010).

Few studies have measured maternal vitamin D status during pregnancy and type I diabetes incidence in the offspring. A nested case-control study in Norway found a more than two-fold odds of type I diabetes in offspring of women in the lowest compared with the highest quartile of serum 25(OH)D late in pregnancy (adjusted OR 2.39, 95% CI 1.07 to 5.31) (Sorensen *et al.*, 2012). In a nested case-control study in Finland, however, maternal 25(OH)D in early pregnancy did not differ between mothers whose children later developed type 1 diabetes and mothers of non-diabetic 'healthy' children of the same age (Miettinen *et al.*, 2012).

34.7.3 Schizophrenia

Enlarged lateral ventricles and reduced cortical thickness of the brain and low plasma concentrations of nerve growth factor and synapsin II are common pathological features in rat pups born to VDD dams and in humans with schizophrenia (Harrison, 1999; Levenson and Figueiroa, 2008). In multiple studies, incidence of schizophrenia is higher in those born in winter or spring (Torrey *et al.*, 1997), at higher latitudes (Saha *et al.*, 2006), and to dark-skinned migrants (Cantor-Graae and Selten, 2005), factors also implicated in hypovitaminosis D (Holick, 1995). In the first study to examine neonatal vitamin D status and schizophrenia risk directly, neonates in the lowest three quintiles of 25(OH)D based on analysis of dried blood samples had a two-fold risk of developing schizophrenia later in life compared with neonates in the fourth quintile (RR 2.0-2.1 for each quintile, 95% CI: 1.3 to 3.5). However, the risk was nearly equally elevated in

neonates in the highest quintile of 25(OH)D (RR 1.71, 95% CI 1.04 to 2.8) (McGrath *et al.*, 2010). The authors speculated that single nucleotide polymorphisms in vitamin D-related genes could result in the U-shaped relationship between gestational vitamin D status and schizophrenia risk.

34.7.4 Multiple sclerosis

Childhood sunlight exposure, vitamin D intake from food and supplements, and serum 25(OH)D are inversely associated with risk of MS, an autoimmune disease (Kampman *et al.*, 2007; Munger *et al.*, 2004, 2006; Van der Mei *et al.*, 2003). Season of birth patterns and latitudinal gradients in MS distribution are evident, with MS patients more likely to be born in the spring (Staples *et al.*, 2010; Willer *et al.*, 2005) and a higher prevalence of disease further from the equator (Simpson *et al.*, 2011). A higher concordance rate of MS among dizygotic twins than non-twin siblings suggests a gestational component of susceptibility (Ebers, 2008). In a large cohort study, mothers of female nurses followed prospectively for more than 20 years completed a questionnaire inquiring about their experiences and diet during pregnancy with their nurse daughters. Risk of MS in the offspring was significantly lower in nurses whose mothers were in the highest quintile of dietary vitamin D intake compared with the lowest (adjusted RR 0.57, 95% CI 0.35 to 0.91). Furthermore, predicted 25(OH)D of the pregnant mothers based on dietary and lifestyle factors was inversely associated with risk of MS in their daughters (P=0.002 for trend) (Mirzaei *et al.*, 2011).

34.8 Recommended intakes and monitoring of vitamin D status in pregnancy

Vitamin D intake recommendations during pregnancy are available from many countries globally. As of 2008, recommendations for vitamin D intake in Western and Eastern Europe ranged from 5-11.3 μg/d, with a median and mode of 10 μg/d (Doets *et al.*, 2008). The most recent World Health Organization/Food and Agriculture Organization guidelines, published in 2004, recommend an intake of 5 μg/d vitamin D during pregnancy (WHO/FAO, 2004). The governments of Australia and New Zealand likewise recommend 5 μg/d vitamin D during pregnancy (Council, 2006). In the United States and Canada, the estimated average requirement and recommended dietary allowance for vitamin D during pregnancy were recently set at 10 μg/d and 15 μg/d, respectively (Ross *et al.*, 2010).

In the majority of countries recommendations for vitamin D intake during pregnancy exceed those for non-pregnant individuals, while in Australia, Austria, Bulgaria, Canada, Germany, Iceland, New Zealand, Romania, Switzerland, and the United States recommendations during pregnancy are equivalent to general recommendations. The dietary reference intakes for vitamin D in the United States and Canada were determined on the basis of an integrated bone health indicator and a simulated dose-response curve considering 25(OH)D ≥40 or 50 nmol/l to meet the needs of 50% and 97.5% of individuals, respectively, assuming minimal sun exposure (Ross *et al.*, 2010). Recommended intakes during pregnancy parallel those for the general population due to insufficiency of evidence on the association of serum 25(OH)D with maternal bone mineral

density or fetal calcium homeostasis and skeletal outcomes. In Australia and New Zealand, the placental transfer of vitamin D and its metabolites was considered 'too small to affect the mother's vitamin D requirement' (NMHRC, 2006).

Recent literature suggests that skeletal craniotabes in newborns are associated with *in utero* VDD (Yorifuji *et al.*, 2008) and that adverse changes to fetal bone morphology may be seen as early as the second trimester of gestation (Mahon *et al.*, 2010). As reviewed in the present chapter, gestational VDD may have lasting effects on multiple other physiological systems in the offspring. Furthermore, the recommended target range of serum 25(OH)D in non-pregnant adults is variably considered to be 80-250 nmol/l (Mulligan *et al.*, 2010), considerably higher than the concentrations on which most recommended intakes were based. A review of recent scientific evidence concluded that circulating maternal 25(OH)D during pregnancy should be 100-150 nmol/l and that intakes of 50 µg/d are necessary to achieve this concentration (Hollis and Wagner, 2011). The United States Endocrine Task Force on Vitamin D reported in 2011 that 15 µg/d may be insufficient to correct VDD in pregnant women and recommends intakes of 37.5-50 µg/d (Holick *et al.*, 2011), while the Canadian Pediatric Society recommends 50 µg/d to maintain vitamin D sufficiency (First Nations, 2007). Due to continuous changes in scientific knowledge, revisions to European and other recommendations are expected (Doets *et al.*, 2008).

Most prenatal vitamins contain 10 µg vitamin D as a result of prior and current guidelines (Hollis *et al.*, 2011), though studies have found an unacceptable prevalence of vitamin D deficiency and insufficiency among pregnant women consuming this dose (Dror *et al.*, 2011; Li *et al.*, 2011). Current prenatal care in most countries does not include monitoring of maternal vitamin D status. In particular for women with risk factors for deficiency, including location of residence far from the equator, winter pregnancy, limited sun exposure and/or regular use of sunscreen, dark skin, obestiy, extensive clothing cover, or malabsorptive disorders, determination of maternal 25(OH)D in early and mid-pregnancy should be considered as a preventative measure in clinical care (Mulligan *et al.*, 2010).

34.9 Supplementation and toxicity in pregnancy

Historically, vitamin D supplementation during pregnancy was considered a risk factor for supravalvular aortic stenosis, a congenital heart defect, in infants (Garcia *et al.*, 1964). This condition was later attributed to Williams Syndrome, a rare genetic disorder characterized by dysmorphic facial features, multiorgan involvement including supravalvular aortic stenosis, and an exaggerated response of circulating 25(OH)D to exogenous vitamin D (Dror and Allen, 2010). Since the 1980s, trials involving regular supplementation with 10-30 µg/d or a single or double dose of 1,500-15,000 µg during the second half of pregnancy have demonstrated no adverse effects (Brooke *et al.*, 1980; Cockburn *et al.*, 1980; Delvin *et al.*, 1986; Kalra *et al.*, 2012; Mallet *et al.*, 1986; Marya *et al.*, 1981; Yu *et al.*, 2009). In the first RCT to include higher doses of continued supplementation, women receiving 100 µg/d vitamin D starting at 12-16 weeks gestation experienced no adverse effects and achieved significantly higher maternal serum 25(OH)D one

month before delivery and at delivery compared with those receiving 50 or 10 µg/d (Hollis *et al.*, 2011). In addition, cord blood 25(OH)D varied significantly by supplement group, with neonates of mothers receiving 100 µg/d achieving the highest levels of circulating 25(OH)D ($P<0.0001$). A biphasic relationship was demonstrated between circulating 25(OH)D and $1,25(OH)_2D$, with circulating levels of 25(OH)D >100 nmol/l required to support maximum $1,25(OH)_2D$ output.

The tolerable upper limit for vitamin D in the United States and Canada is set at 100 µg/d for pregnant and non-pregnant individuals, based on evidence of hypercalcemia at intakes above 250 µg/d in non-pregnant adults and lack of data supporting a different tolerable upper limit specific to pregnancy (Ross *et al.*, 2010). Intake of 100 µg/d is estimated to result in circulating 25(OH)D concentrations of approximately 150 nmol/l (Heaney *et al.*, 2003). In the RCT involving supplementation of pregnant women with 100 µg/d vitamin D, evidence of toxicity was not observed despite circulating 25(OH)D exceeding 225 nmol/l in three cases (Hollis *et al.*, 2011). Supplementation was initiated after 12 weeks gestation, such that additional research is necessary to ascertain safety of this dose prior to the twelfth week. Most experts agree that supplementation with 25-50 µg/d vitamin D during pregnancy is safe (ACOG, 2011).

34.10 Conclusion

The prevalence of vitamin D deficiency during pregnancy is concerning in light of potential implications on multiple maternal, fetal, and postnatal health consequences. While recommendations for vitamin D intake during pregnancy have been revised or are undergoing revision in many countries to account for recent evidence of elevated needs during pregnancy, in most cases existing recommendations are insufficient to raise circulating 25(OH)D to acceptable concentrations. Vitamin D supplementation studies during pregnancy have demonstrated short-term safety and efficacy, though additional research is necessary to verify long-term safety and to establish a clinical protocol for monitoring and maintaining vitamin D adequacy during pregnancy.

References

ACOG, 2011. ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy. Obstet Gynecol 118, 197-198.

Akcakus, M., Koklu, E., Budak, N., Kula, M., Kurtoglu, S. and Koklu, S., 2006. The relationship between birthweight, 25-hydroxyvitamin D concentrations and bone mineral status in neonates. Ann Trop Paediatr 26, 267-275.

Aleyasin, A., Hosseini, M.A., Mahdavi, A., Safdarian, L., Fallahi, P., Mohajeri, M.R., Abbasi, M. and Esfahani, F., 2011. Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology. Eur J Obstet Gynecol Reprod Biol 159, 132-137.

Anifandis, G.M., Dafopoulos, K., Messini, C.I., Chalvatzas, N., Liakos, N., Pournaras, S. and Messinis, I.E., 2010. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. Reprod Biol Endocrinol 8, 91.

August, P., Marcaccio, B., Gertner, J.M., Druzin, M.L., Resnick, L.M. and Laragh, J.H., 1992. Abnormal 1,25-dihydroxyvitamin D metabolism in preeclampsia. Am J Obstet Gynecol 166, 1295-1299.

Baker, A.M., Haeri, S., Camargo Jr., C.A., Espinola, J.A. and Stuebe, A.M., 2010. A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. J Clin Endocrinol Metab 95, 5105-5109.

Baker, A.M., Haeri, S., Camargo Jr., C.A., Stuebe, A.M. and Boggess, K.A., 2011. A nested case-control study of first-trimester maternal vitamin D status and risk for spontaneous preterm birth. Am J Perinatol 28, 667-672.

Baker, A.M., Haeri, S., Camargo Jr., C.A., Stuebe, A.M. and Boggess, K.A., 2012. First-trimester maternal vitamin D status and risk for gestational diabetes (GDM) a nested case-control study. Diabetes Metab Res Rev 28, 164-168.

Barker, D.J., Eriksson, J.G., Forsen, T. and Osmond, C., 2002. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol 31, 1235-1239.

Begum, K.S., Sachchithanantham, K. and De Somsubhra, S., 2011. Maternal obesity and pregnancy outcome. Clin Exp Obstet Gynecol 38, 14-20.

Belderbos, M.E., Houben, M.L., Wilbrink, B., Lentjes, E., Bloemen, E.M., Kimpen, J.L., Rovers, M. and Bont, L., 2011. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics 127, e1513-1520.

Bikle, D.D., Gee, E., Halloran, B. and Haddad, J.G., 1984. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. J Clin Invest 74, 1966-1971.

Bikle, D.D., Gee, E., Halloran, B., Kowalski, M.A., Ryzen, E. and Haddad, J.G., 1986. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab 63, 954-959.

Bikle, D.D., Halloran, B.P., Harris, S.T. and Portale, A.A., 1992. Progestin antagonism of estrogen stimulated 1,25-dihydroxyvitamin D levels. J Clin Endocrinol Metab 75, 519-523.

Bikle, D.D., Siiteri, P.K., Ryzen, E. and Haddad, J.G., 1985. Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels. J Clin Endocrinol Metab 61, 969-975.

Bodnar, L.M., Catov, J.M., Simhan, H.N., Holick, M.F., Powers, R.W. and Roberts, J.M., 2007. Maternal vitamin D deficiency increases the risk of preeclampsia. J Clin Endocrinol Metab 92, 3517-3522.

Bodnar, L.M., Catov, J.M., Zmuda, J.M., Cooper, M.E., Parrott, M.S., Roberts, J.M., Marazita, M.L. and Simhan, H.N., 2010. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. J Nutr 140, 999-1006.

Bodnar, L.M., Krohn, M.A. and Simhan, H.N., 2009. Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. J Nutr 139, 1157-1161.

Boggess, K.A., Espinola, J.A., Moss, K., Beck, J., Offenbacher, S. and Camargo Jr., C.A., 2011. Vitamin D status and periodontal disease among pregnant women. J Periodontol 82, 195-200.

Bouillon, R., Van Assche, F.A., Van Baelen, H., Heyns, W. and De Moor, P., 1981. Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D3. Significance of the free 1,25-dihydroxyvitamin D3 concentration. J Clin Invest 67, 589-596.

Bowyer, L., Catling-Paull, C., Diamond, T., Homer, C., Davis, G. and Craig, M.E., 2009. Vitamin D, PTH and calcium levels in pregnant women and their neonates. Clin Endocrinol (Oxf) 70, 372-377.

Brannon, P.M. and Picciano, M.F., 2011. Vitamin D in pregnancy and lactation in humans. Annu Rev Nutr 31, 89-115.

Brekke, H.K. and Ludvigsson, J., 2007. Vitamin D supplementation and diabetes-related autoimmunity in the ABIS study. Pediatr Diabetes 8, 11-14.

Brooke, O.G., Brown, I.R., Bone, C.D., Carter, N.D., Cleeve, H.J., Maxwell, J.D., Robinson, V.P. and Winder, S.M., 1980. Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. Br Med J 280, 751-754.

Brunvand, L., Shah, S.S., Bergstrom, S. and Haug, E., 1998. Vitamin D deficiency in pregnancy is not associated with obstructed labor. A study among Pakistani women in Karachi. Acta Obstet Gynecol Scand 77, 303-306.

Camargo Jr., C.A., Ingham, T., Wickens, K., Thadhani, R., Silvers, K.M., Epton, M.J., Town, G.I., Pattemore, P.K., Espinola, J.A. and Crane, J., 2011. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. Pediatrics 127, e180-187.

Camargo Jr., C.A., Rifas-Shiman, S.L., Litonjua, A.A., Rich-Edwards, J.W., Weiss, S.T., Gold, D.R., Kleinman, K. and Gillman, M.W., 2007. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr 85, 788-795.

Cantor-Graae, E. and Selten, J.P., 2005. Schizophrenia and migration: a meta-analysis and review. Am J Psychiatry 162, 12-24.

Chi, A., Wildfire, J., McLoughlin, R., Wood, R.A., Bloomberg, G.R., Kattan, M., Gergen, P., Gold, D.R., Witter, F., Chen, T., Holick, M., Visness, C., Gern, J. and O'Connor, G.T., 2011. Umbilical cord plasma 25-hydroxyvitamin D concentration and immune function at birth: the Urban Environment and Childhood Asthma study. Clin Exp Allergy 41, 842-850.

Clifton-Bligh, R.J., McElduff, P. and McElduff, A., 2008. Maternal vitamin D deficiency, ethnicity and gestational diabetes. Diabet Med 25, 678-684.

Cockburn, F., Belton, N.R., Purvis, R.J., Giles, M.M., Brown, J.K., Turner, T.L., Wilkinson, E.M., Forfar, J.O., Barrie, W.J., McKay, G.S. and Pocock, S.J., 1980. Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. Br Med J 281, 11-14.

Congdon, P., Horsman, A., Kirby, P.A., Dibble, J. and Bashir, T., 1983. Mineral content of the forearms of babies born to Asian and white mothers. Br Med J (Clin Res Ed) 286, 1233-1235.

Council, N.M.H.a.R., 2006. Nutrient reference values for Australia and New Zealand including recommended dietary intakes, Australian Government Department of Health and Ageing.

Cui, X., McGrath, J.J., Burne, T.H., Mackay-Sim, A. and Eyles, D.W., 2007. Maternal vitamin D depletion alters neurogenesis in the developing rat brain. Int J Dev Neurosci 25, 227-232.

Davis, L.M., Chang, S.C., Mancini, J., Nathanson, M.S., Witter, F.R. and O'Brien, K.O., 2010. Vitamin D insufficiency is prevalent among pregnant African American adolescents. J Pediatr Adolesc Gynecol 23, 45-52.

Dawson-Hughes, B., Heaney, R.P., Holick, M.F., Lips, P., Meunier, P.J. and Vieth, R., 2005. Estimates of optimal vitamin D status. Osteoporos Int 16, 713-716.

De-Regil, L.M., Palacios, C., Ansary, A., Kulier, R. and Pena-Rosas, J.P., 2012. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2, CD008873.

Deluca, H.F. and Cantorna, M.T., 2001. Vitamin D: its role and uses in immunology. FASEB J 15, 2579-2585.

Delvin, E.E., Salle, B.L., Glorieux, F.H., Adeleine, P. and David, L.S., 1986. Vitamin D supplementation during pregnancy: effect on neonatal calcium homeostasis. J Pediatr 109, 328-334.

Devereux, G., Litonjua, A.A., Turner, S.W., Craig, L.C., McNeill, G., Martindale, S., Helms, P.J., Seaton, A. and Weiss, S.T., 2007. Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr 85, 853-859.

Dick, I.M., Prince, R.L., Kelly, J.J. and Ho, K.K., 1995. Oestrogen effects on calcitriol levels in post-menopausal women: a comparison of oral versus transdermal administration. Clin Endocrinol 43, 219-224.

Doets, E.L., de Wit, L.S., Dhonukshe-Rutten, R.A., Cavelaars, A.E., Raats, M.M., Timotijevic, L., Brzozowska, A., Wijnhoven, T.M., Pavlovic, M., Totland, T.H., Andersen, L.F., Ruprich, J., Pijls, L.T., Ashwell, M., Lambert, J.P., van 't Veer, P. and de Groot, L.C., 2008. Current micronutrient recommendations in Europe: towards understanding their differences and similarities. Eur J Nutr 47 Suppl 1, 17-40.

Dror, D.K., 2011. Vitamin D status during pregnancy: maternal, fetal, and postnatal outcomes. Curr Opin Obstet Gynecol 23, 422-426.

Dror, D.K. and Allen, L.H., 2010. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. Nutr Rev 68, 465-477.

Dror, D.K., King, J.C., Durand, D.J. and Allen, L.H., 2011. Association of modifiable and nonmodifiable factors with vitamin D status in pregnant women and neonates in Oakland, CA. J Am Diet Assoc 111, 111-116.

Dunlop, A.L., Taylor, R.N., Tangpricha, V., Fortunato, S. and Menon, R., 2011. Maternal vitamin D, folate, and polyunsaturated fatty acid status and bacterial vaginosis during pregnancy. Infect Dis Obstet Gynecol 2011, 216-217.

Ebers, G.C., 2008. Environmental factors and multiple sclerosis. Lancet Neurol 7, 268-277.

Erkkola, M., Kaila, M., Nwaru, B.I., Kronberg-Kippila, C., Ahonen, S., Nevalainen, J., Veijola, R., Pekkanen, J., Ilonen, J., Simell, O., Knip, M. and Virtanen, S.M., 2009. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. Clin Exp Allergy 39, 875-882.

Ertl, R., Yu, C.K., Samaha, R., Akolekar, R. and Nicolaides, K.H., 2012. maternal serum vitamin d at 11-13 weeks in pregnancies delivering small for gestational age neonates. Fetal Diagn Ther. 31(2), 103-108.

Evans, K.N., Bulmer, J.N., Kilby, M.D. and Hewison, M., 2004. Vitamin D and placental-decidual function. J Soc Gynecol Investig 11, 263-271.

Eyles, D., Brown, J., Mackay-Sim, A., McGrath, J. and Feron, F., 2003. Vitamin D3 and brain development. Neuroscience 118, 641-653.

Eyles, D., Burne, T. and McGrath, J., 2011. Vitamin D in fetal brain development. Semin Cell Dev Biol 22, 629-636.

Farrant, H.J., Krishnaveni, G.V., Hill, J.C., Boucher, B.J., Fisher, D.J., Noonan, K., Osmond, C., Veena, S.R. and Fall, C.H., 2009. Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. Eur J Clin Nutr 63, 646-652.

First Nations, I.a.M.H.C., 2007. Vitamin D supplementation: Recommendations for Canadian mothers and infants, Canadian Paediatric Society.

Fronczak, C.M., Baron, A.E., Chase, H.P., Ross, C., Brady, H.L., Hoffman, M., Eisenbarth, G.S., Rewers, M. and Norris, J.M., 2003. *in utero* dietary exposures and risk of islet autoimmunity in children. Diabetes Care 26, 3237-3242.

Gale, C.R., Robinson, S.M., Harvey, N.C., Javaid, M.K., Jiang, B., Martyn, C.N., Godfrey, K.M. and Cooper, C., 2008. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 62, 68-77.

Garcia, R.E., Friedman, W.F., Kaback, M.M. and Rowe, R.D., 1964. Idiopathic Hypercalcemia and Supravalvular Aortic Stenosis. Documentation of a New Syndrome. N Engl J Med 271, 117-120.

Grundmann, M. and von Versen-Hoynck, F., 2011. Vitamin D – roles in women's reproductive health? Reprod Biol Endocrinol 9, 146.

Haddad Jr., J.G. and Walgate, J., 1976. Radioimmunoassay of the binding protein for vitamin D and its metabolites in human serum: concentrations in normal subjects and patients with disorders of mineral homeostasis. J Clin Invest 58, 1217-1222.

Halhali, A., Bourges, H., Carrillo, A. and Garabedian, M., 1995. Lower circulating insulin-like growth factor I and 1,25-dihydroxyvitamin D levels in preeclampsia. Rev Invest Clin 47, 259-266.

Halhali, A., Tovar, A.R., Torres, N., Bourges, H., Garabedian, M. and Larrea, F., 2000. Preeclampsia is associated with low circulating levels of insulin-like growth factor I and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. J Clin Endocrinol Metab 85, 1828-1833.

Halhali, A., Villa, A.R., Madrazo, E., Soria, M.C., Mercado, E., Diaz, L., Avila, E., Garabedian, M. and Larrea, F., 2004. Longitudinal changes in maternal serum 1,25-dihydroxyvitamin D and insulin like growth factor I levels in pregnant women who developed preeclampsia: comparison with normotensive pregnant women. J Steroid Biochem Mol Biol 89-90, 553-556.

Harrison, P.J., 1999. The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122 (Pt 4), 593-624.

Heaney, R.P., Davies, K.M., Chen, T.C., Holick, M.F. and Barger-Lux, M.J., 2003. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 77, 204-210.

Hensel, K.J., Randis, T.M., Gelber, S.E. and Ratner, A.J., 2011. Pregnancy-specific association of vitamin D deficiency and bacterial vaginosis. Am J Obstet Gynecol 204, 41 e41-49.

Holick, M.F., 1995. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 61, 638S-645S.

Holick, M.F., 2007. Vitamin D deficiency. N Engl J Med 357, 266-281.

Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H. and Weaver, C.M., 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96, 1911-1930.

Hollis, B.W., 2005. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 135, 317-322.

Hollis, B.W., Johnson, D., Hulsey, T.C., Ebeling, M. and Wagner, C.L., 2011. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res 26, 2341-2357.

Hollis, B.W. and Wagner, C.L., 2011. Vitamin D requirements and supplementation during pregnancy. Curr Opin Endocrinol Diabetes Obes 18, 371-375.

Hypponen, E., 2011. Preventing vitamin D deficiency in pregnancy: importance for the mother and child. Ann Nutr Metab 59, 28-31.

Kalra, P., Das, V., Agarwal, A., Kumar, M., Ramesh, V., Bhatia, E., Gupta, S., Singh, S., Saxena, P. and Bhatia, V., 2012. Effect of vitamin D supplementation during pregnancy on neonatal mineral homeostasis and anthropometry of the newborn and infant. Br J Nutr 108, 1052-1058.

Kampman, M.T., Wilsgaard, T. and Mellgren, S.I., 2007. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. J Neurol 254, 471-477.

Ko, P., Burkert, R., McGrath, J. and Eyles, D., 2004. Maternal vitamin D3 deprivation and the regulation of apoptosis and cell cycle during rat brain development. Brain Res Dev Brain Res 153, 61-68.

Kovacs, C.S., 2008. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 88, 520S-528S.

Lanham-New, S.A., Buttriss, J.L., Miles, L.M., Ashwell, M., Berry, J.L., Boucher, B.J., Cashman, K.D., Cooper, C., Darling, A.L., Francis, R.M., Fraser, W.D., de Groot, C.P., Hypponen, E., Kiely, M., Lamberg-Allardt, C., Macdonald, H.M., Martineau, A.R., Masud, T., Mavroeidi, A., Nowson, C., Prentice, A., Stone, E.M., Reddy, S., Vieth, R. and Williams, C.M., 2011. Proceedings of the Rank Forum on Vitamin D. Br J Nutr 105, 144-156.

Lau, S.L., Gunton, J.E., Athayde, N.P., Byth, K. and Cheung, N.W., 2011. Serum 25-hydroxyvitamin D and glycated haemoglobin levels in women with gestational diabetes mellitus. Med J Aust 194, 334-337.

Leffelaar, E.R., Vrijkotte, T.G. and van Eijsden, M., 2010. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. Br J Nutr 104, 108-117.

Lerchbaum, E. and Obermayer-Pietsch, B.R., 2012. Vitamin D and fertility-a systematic review. Eur J Endocrinol 166(5), 765-778.

Levenson, C.W. and Figueiroa, S.M., 2008. Gestational vitamin D deficiency: long-term effects on the brain. Nutr Rev 66, 726-729.

Li, W., Green, T.J., Innis, S.M., Barr, S.I., Whiting, S.J., Shand, A. and von Dadelszen, P., 2011. Suboptimal vitamin D levels in pregnant women despite supplement use. Can J Public Health 102, 308-312.

Litonjua, A.A. and Weiss, S.T., 2007. Is vitamin D deficiency to blame for the asthma epidemic? J Allergy Clin Immunol 120, 1031-1035.

Liu, N.Q. and Hewison, M., 2012. Vitamin D, the placenta and pregnancy. Arch Biochem Biophys 523(1), 37-47.

Lucas, R.M., Ponsonby, A.L., Pasco, J.A. and Morley, R., 2008. Future health implications of prenatal and early-life vitamin D status. Nutr Rev 66, 710-720.

Maghbooli, Z., Hossein-Nezhad, A., Karimi, F., Shafaei, A.R. and Larijani, B., 2008. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev 24, 27-32.

Mahon, P., Harvey, N., Crozier, S., Inskip, H., Robinson, S., Arden, N., Swaminathan, R., Cooper, C. and Godfrey, K., 2010. Low maternal vitamin D status and fetal bone development: cohort study. J Bone Miner Res 25, 14-19.

Mallet, E., Gugi, B., Brunelle, P., Henocq, A., Basuyau, J.P. and Lemeur, H., 1986. Vitamin D supplementation in pregnancy: a controlled trial of two methods. Obstet Gynecol 68, 300-304.

Marjamaki, L., Niinisto, S., Kenward, M.G., Uusitalo, L., Uusitalo, U., Ovaskainen, M.L., Kronberg-Kippila, C., Simell, O., Veijola, R., Ilonen, J., Knip, M. and Virtanen, S.M., 2010. Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. Diabetologia 53, 1599-1607.

Marya, R.K., Rathee, S., Lata, V. and Mudgil, S., 1981. Effects of vitamin D supplementation in pregnancy. Gynecol Obstet Invest 12, 155-161.

Marya, R.K., Rathee, S. and Manrow, M., 1987. Effect of calcium and vitamin D supplementation on toxaemia of pregnancy. Gynecol Obstet Invest 24, 38-42.

McCann, J.C. and Ames, B.N., 2008. Is there convincing biological or behavioral evidence linking vitamin D deficiency to brain dysfunction? FASEB J 22, 982-1001.

McGrath, J.J., Eyles, D.W., Pedersen, C.B., Anderson, C., Ko, P., Burne, T.H., Norgaard-Pedersen, B., Hougaard, D.M. and Mortensen, P.B., 2010. Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. Arch Gen Psychiatry 67, 889-894.

McLeod, D.S., Warner, J.V., Henman, M., Cowley, D., Gibbons, K. and McIntyre, H.D., 2012. Associations of serum vitamin D concentrations with obstetric glucose metabolism in a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study cohort. Diabet Med. 29(8), e199-204.

Mehta, S., Giovannucci, E., Mugusi, F.M., Spiegelman, D., Aboud, S., Hertzmark, E., Msamanga, G.I., Hunter, D. and Fawzi, W.W., 2010. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. PLoS One 5, e8770.

Mehta, S., Hunter, D.J., Mugusi, F.M., Spiegelman, D., Manji, K.P., Giovannucci, E.L., Hertzmark, E., Msamanga, G.I. and Fawzi, W.W., 2009. Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania. J Infect Dis 200, 1022-1030.

Merewood, A., Mehta, S.D., Chen, T.C., Bauchner, H. and Holick, M.F., 2009. Association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab 94, 940-945.

Miettinen, M.E., Reinert, L., Kinnunen, L., Harjutsalo, V., Koskela, P., Surcel, H.M., Lamberg-Allardt, C. and Tuomilehto, J., 2012. Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. Diabetologia 55(5), 1291-1294.

Mirzaei, F., Michels, K.B., Munger, K., O'Reilly, E., Chitnis, T., Forman, M.R., Giovannucci, E., Rosner, B. and Ascherio, A., 2011. Gestational vitamin D and the risk of multiple sclerosis in offspring. Ann Neurol 70, 30-40.

Miyake, Y., Sasaki, S., Tanaka, K. and Hirota, Y., 2010. Dairy food, calcium and vitamin D intake in pregnancy, and wheeze and eczema in infants. Eur Respir J 35, 1228-1234.

Morales, E., Romieu, I., Guerra, S., Ballester, F., Rebagliato, M., Vioque, J., Tardon, A., Rodriguez Delhi, C., Arranz, L., Torrent, M., Espada, M., Basterrechea, M. and Sunyer, J., 2012. Maternal vitamin d status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. Epidemiology 23, 64-71.

Morley, R., Carlin, J.B., Pasco, J.A. and Wark, J.D., 2006. Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size. J Clin Endocrinol Metab 91, 906-912.

Morley, R., Carlin, J.B., Pasco, J.A., Wark, J.D. and Ponsonby, A.L., 2009. Maternal 25-hydroxyvitamin D concentration and offspring birth size: effect modification by infant VDR genotype. Eur J Clin Nutr 63, 802-804.

Mostello, D., Catlin, T.K., Roman, L., Holcomb Jr., W.L. and Leet, T., 2002. Preeclampsia in the parous woman: who is at risk? Am J Obstet Gynecol 187, 425-429.

Mulligan, M.L., Felton, S.K., Riek, A.E. and Bernal-Mizrachi, C., 2010. Implications of vitamin D deficiency in pregnancy and lactation. Am J Obstet Gynecol 202, 429 e421-429.

Munger, K.L., Levin, L.I., Hollis, B.W., Howard, N.S. and Ascherio, A., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296, 2832-2838.

Munger, K.L., Zhang, S.M., O'Reilly, E., Hernan, M.A., Olek, M.J., Willett, W.C. and Ascherio, A., 2004. Vitamin D intake and incidence of multiple sclerosis. Neurology 62, 60-65.

Namgung, R., Tsang, R.C., Lee, C., Han, D.G., Ho, M.L. and Sierra, R.I., 1998. Low total body bone mineral content and high bone resorption in Korean winter-born versus summer-born newborn infants. J Pediatr 132, 421-425.

NMHRC, 2006. Nutrient reference values for Australia and New Zealand including recommended dietary intakes. Australian Government Department of Health and Ageing, National Medical Health and Research Council.

Novakovic, B., Sibson, M., Ng, H.K., Manuelpillai, U., Rakyan, V., Down, T., Beck, S., Fournier, T., Evain-Brion, D., Dimitriadis, E., Craig, J.M., Morley, R. and Saffery, R., 2009. Placenta-specific methylation of the vitamin D 24-hydroxylase gene: implications for feedback autoregulation of active vitamin D levels at the fetomaternal interface. J Biol Chem 284, 14838-14848.

Ozkan, S., Jindal, S., Greenseid, K., Shu, J., Zeitlian, G., Hickmon, C. and Pal, L., 2010. Replete vitamin D stores predict reproductive success following *in vitro* fertilization. Fertil Steril 94, 1314-1319.

Papapetrou, P.D., 2010. The interrelationship of serum 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in pregnancy at term: a meta-analysis. Hormones (Athens) 9, 136-144.

Parlea, L., Bromberg, I.L., Feig, D.S., Vieth, R., Merman, E. and Lipscombe, L.L., 2012. Association between serum 25-hydroxyvitamin D in early pregnancy and risk of gestational diabetes mellitus. Diabet Med 29(7), e25-32.

Powe, C.E., Seely, E.W., Rana, S., Bhan, I., Ecker, J., Karumanchi, S.A. and Thadhani, R., 2010. First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. Hypertension 56, 758-763.

Prentice, A., Jarjou, L.M., Goldberg, G.R., Bennett, J., Cole, T.J. and Schoenmakers, I., 2009. Maternal plasma 25-hydroxyvitamin D concentration and birthweight, growth and bone mineral accretion of Gambian infants. Acta Paediatr 98, 1360-1362.

Ramos-Lopez, E., Kahles, H., Weber, S., Kukic, A., Penna-Martinez, M., Badenhoop, K. and Louwen, F., 2008. Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms. Diabetes Obes Metab 10, 683-685.

Ritchie, L.D., Fung, E.B., Halloran, B.P., Turnlund, J.R., Van Loan, M.D., Cann, C.E. and King, J.C., 1998. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr 67, 693-701.

Roberts, J.M. and Lain, K.Y., 2002. Recent Insights into the pathogenesis of pre-eclampsia. Placenta 23, 359-372.

Robinson, C.J., Alanis, M.C., Wagner, C.L., Hollis, B.W. and Johnson, D.D., 2010. Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. Am J Obstet Gynecol 203, 366 e361-366.

Rochat, M.K., Ege, M.J., Plabst, D., Steinle, J., Bitter, S., Braun-Fahrlander, C., Dalphin, J.C., Riedler, J., Roponen, M., Hirvonen, M.R., Buchele, G., Renz, H., Lauener, R., Krauss-Etschmann, S. and von Mutius, E., 2010. Maternal vitamin D intake during pregnancy increases gene expression of ILT3 and ILT4 in cord blood. Clin Exp Allergy 40, 786-794.

Rojansky, N., Brzezinski, A. and Schenker, J.G., 1992. Seasonality in human reproduction: an update. Hum Reprod 7, 735-745.

Ross, A.C., Taylor, C.L., Yaktine, A.L. and Del Valle, H.B., 2010. Dietary reference intakes for calcium and vitamin D, Institute of Medicine, Washington D.C., USA.

Saha, S., Chant, D.C., Welham, J.L. and McGrath, J.J., 2006. The incidence and prevalence of schizophrenia varies with latitude. Acta Psychiatr Scand 114, 36-39.

Saito, S. and Sakai, M., 2003. Th1/Th2 balance in preeclampsia. J Reprod Immunol 59, 161-173.

Savvidou, M.D., Makgoba, M., Castro, P.T., Akolekar, R. and Nicolaides, K.H., 2012. First-trimester maternal serum vitamin D and mode of delivery. Br J Nutr 108(11), 1972-1975.

Seely, E.W., Wood, R.J., Brown, E.M. and Graves, S.W., 1992. Lower serum ionized calcium and abnormal calciotropic hormone levels in preeclampsia. J Clin Endocrinol Metab 74, 1436-1440.

Shand, A.W., Nassar, N., Von Dadelszen, P., Innis, S.M. and Green, T.J., 2010. Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. BJOG 117, 1593-1598.

Simpson Jr., S., Blizzard, L., Otahal, P., Van der Mei, I. and Taylor, B., 2011. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. J Neurol Neurosurg Psychiatry 82, 1132-1141.

Soheilykhah, S., Mojibian, M., Rashidi, M., Rahimi-Saghand, S. and Jafari, F., 2010. Maternal vitamin D status in gestational diabetes mellitus. Nutr Clin Pract 25, 524-527.

Sorensen, I.M., Joner, G., Jenum, P.A., Eskild, A., Torjesen, P.A. and Stene, L.C., 2012. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. Diabetes 61, 175-178.

Staples, J., Ponsonby, A.L. and Lim, L., 2010. Low maternal exposure to ultraviolet radiation in pregnancy, month of birth, and risk of multiple sclerosis in offspring: longitudinal analysis. BMJ 340, c1640.

Swamy, G.K., Garrett, M.E., Miranda, M.L. and Ashley-Koch, A.E., 2011. Maternal vitamin D receptor genetic variation contributes to infant birthweight among black mothers. Am J Med Genet A 155A, 1264-1271.

Takiishi, T., Gysemans, C., Bouillon, R. and Mathieu, C., 2010. Vitamin D and diabetes. Endocrinol Metab Clin North Am 39, 419-446.

TePoel, M.R., Saftlas, A.F. and Wallis, A.B., 2011. Association of seasonality with hypertension in pregnancy: a systematic review. J Reprod Immunol 89, 140-152.

Torrey, E.F., Miller, J., Rawlings, R. and Yolken, R.H., 1997. Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. Schizophr Res 28, 1-38.

Urrutia, R.P. and Thorp, J.M., 2012. Vitamin D in pregnancy: current concepts. Curr Opin Obstet Gynecol 24(2), 57-64.

Van den Berg, G., van Eijsden, M., Vrijkotte, T.G. and Gemke, R.J., 2012. Suboptimal maternal vitamin D status and low education level as determinants of small-for-gestational-age birth weight. Eur J Nutr, 52, 273-279.

Van der Mei, I.A., Ponsonby, A.L., Dwyer, T., Blizzard, L., Simmons, R., Taylor, B.V., Butzkueven, H. and Kilpatrick, T., 2003. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ 327, 316.

Van Hoof, H.J., de Sevaux, R.G., van Baelen, H., Swinkels, L.M., Klipping, C., Ross, H.A. and Sweep, C.G., 2001. Relationship between free and total 1,25-dihydroxyvitamin D in conditions of modified binding. Eur J Endocrinol 144, 391-396.

Viljakainen, H.T., Korhonen, T., Hytinantti, T., Laitinen, E.K., Andersson, S., Makitie, O. and Lamberg-Allardt, C., 2011. Maternal vitamin D status affects bone growth in early childhood – a prospective cohort study. Osteoporos Int 22, 883-891.

Viljakainen, H.T., Saarnio, E., Hytinantti, T., Miettinen, M., Surcel, H., Makitie, O., Andersson, S., Laitinen, K. and Lamberg-Allardt, C., 2010. Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab 95, 1749-1757.

Weiler, H., Fitzpatrick-Wong, S., Veitch, R., Kovacs, H., Schellenberg, J., McCloy, U. and Yuen, C.K., 2005. Vitamin D deficiency and whole-body and femur bone mass relative to weight in healthy newborns. CMAJ 172, 757-761.

Whitehouse, A.J., Holt, B.J., Serralha, M., Holt, P.G., Kusel, M.M. and Hart, P.H., 2012. Maternal serum vitamin d levels during pregnancy and offspring neurocognitive development. Pediatrics 129, 485-493.

WHO/FAO, 2004. Vitamin and mineral requirements in human nutrition, Wrold Health Organization and Food and Agriculture Organization of the United Nations, Geneva and Rome, Italy.

Willer, C.J., Dyment, D.A., Sadovnick, A.D., Rothwell, P.M., Murray, T.J. and Ebers, G.C., 2005. Timing of birth and risk of multiple sclerosis: population based study. BMJ 330, 120.

Yorifuji, J., Yorifuji, T., Tachibana, K., Nagai, S., Kawai, M., Momoi, T., Nagasaka, H., Hatayama, H. and Nakahata, T., 2008. Craniotabes in normal newborns: the earliest sign of subclinical vitamin D deficiency. J Clin Endocrinol Metab 93, 1784-1788.

Yu, C.K., Ertl, R., Skyfta, E., Akolekar, R. and Nicolaides, K.H., 2012. Maternal serum vitamin D levels at 11-13 weeks of gestation in preeclampsia. J Hum Hypertens 27(2), 115-118.

Yu, C.K., Sykes, L., Sethi, M., Teoh, T.G. and Robinson, S., 2009. Vitamin D deficiency and supplementation during pregnancy. Clin Endocrinol 70, 685-690.

Zehnder, D., Evans, K.N., Kilby, M.D., Bulmer, J.N., Innes, B.A., Stewart, P.M. and Hewison, M., 2002. The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua. Am J Pathol 161, 105-114.

Zhang, C., Qiu, C., Hu, F.B., David, R.M., van Dam, R.M., Bralley, A. and Williams, M.A., 2008. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PLoS One 3, e3753.

Key facts

- Low serum 25(OH)vitamin D concentrations are linked to an increased risk of many chronic diseases in observational studies.
- Results of observational studies and randomized clinical trials of nutrients and supplements in relation to chronic disease outcomes have often been divergent.
- Correlation does not prove causation in associational studies due to the potential for confounding and other biases.
- Previous randomized trials of supplemental vitamin D have generally tested low doses of vitamin D and have not included prespecified nonskeletal outcomes.
- Large-scale randomized trials of moderate- or high-dose vitamin D are needed to evaluate whether supplementation can reduce the risk of cancer, cardiovascular disease, and other nonskeletal health outcomes.

Summary points

- It is not yet known whether supplemental vitamin D plays a role in preventing cancer and cardiovascular disease (CVD).
- The ongoing *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL) is a 5-year, randomized, double-blind, placebo-controlled clinical trial among 20,000 US men and woman testing whether supplemental vitamin D (2,000 IU/day) is effective for the primary prevention of cancer and CVD.
- VITAL is the first – and, to date, only – large-scale trial of supplemental vitamin D at a dose sufficient to produce meaningful changes in vitamin D blood levels and designed to assess cancer and CVD as the primary outcomes under study.
- In ancillary studies, VITAL is also examining effects of vitamin D on risk of diabetes, hypertension; cognitive decline; depression; fractures; falls; respiratory disorders; infections; anemia; autoimmune disorders, and other outcomes.
- The results of VITAL are expected to shape clinical and public health guidelines regarding the use of supplemental vitamin D for the primary prevention of cancer, CVD, and other health conditions.

35. Design and rationale of the *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL)

S.S. Bassuk[1] and J.E. Manson[1,2]
[1]Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 900 Commonwealth Avenue East, Boston, MA 02215, USA;
[2]Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA; jmanson@rics.bwh.harvard.edu

Abstract

Although data from laboratory studies, observational research, and secondary prevention trials suggest that vitamin D may protect against the development of cancer and CVD, primary prevention trials with adequate dosing in general populations are needed to test this hypothesis. The *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL) is an ongoing randomized, double-blind, placebo-controlled, 2×2 factorial trial of 5 years of supplemental vitamin D (vitamin D3 [cholecalciferol], 2,000 IU/day) and marine omega-3 fatty acids (Omacor® fish oil, EPA + DHA, 1 g/day) in the primary prevention of cancer and CVD in a racially/ethnically diverse population of 20,000 US men aged ≥50 and women aged ≥55. Blood samples will be collected from at least 16,000 participants at baseline, with follow-up blood collection in about 6,000 participants. Annual questionnaires assess compliance with treatment, use of non-study supplements, occurrence of endpoints, and risk factors for cancer and CVD. Self-reported endpoints are confirmed by medical record review by physicians blinded to treatment assignment, and deaths are ascertained through national registries and other sources. Ancillary investigations are assessing whether the study agents affect risk for diabetes and glucose intolerance; hypertension; cognitive decline; depression; osteoporosis and fracture; physical disability and falls; asthma and other respiratory diseases; infections; kidney disease; anemia; eye problems such as macular degeneration and dry eye syndrome; and autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, and thyroid diseases. The results of the trial are expected to inform clinical and public health guidelines regarding the use of vitamin D for the primary prevention of cancer, CVD, and other chronic diseases.

Keywords: cancer, cardiovascular disease, cholecalciferol, docosahexaenoic acid, eicosapentaenoic acid, fish oil, marine omega-3 fatty acids, primary prevention, randomized controlled trial, vitamin D

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3_35, © Wageningen Academic Publishers 2013

Abbrevations

25(OH)D	25-hydroxyvitamin D
CABG	Coronary artery bypass grafting
CTSC	Clinical and Translational Science Center
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
MI	Myocardial infarction
PCI	Percutaneous coronary intervention
RDA	Recommended dietary allowance
RR	Rate ratio
VITAL	*VIT*amin D and Omeg*A*-3 Tria*L*

35.1 Introduction

Whether vitamin D plays a role in preventing cancer (Manson *et al.*, 2011) or CVD (Shapses and Manson, 2011) is unclear. Data from laboratory investigations (Deeb *et al.*, 2007; Krishnan and Feldman, 2011; Manson and Bassuk, 2009), ecologic studies (Grant, 2002, 2003; Mizoue, 2004; Zittermann *et al.*, 2005), epidemiologic studies (Abbas *et al.*, 2008; Anderson *et al.*, 2010; Bertone-Johnson *et al.*, 2005; Dobnig *et al.*, 2008; Freedman *et al.*, 2007; Giovannucci *et al.*, 2008; Giovannucci *et al.*, 2006; Gorham *et al.*, 2005, 2007; Lowe *et al.*, 2005; Pilz *et al.*, 2008; Wang *et al.*, 2008), and secondary analyses of small randomized trials (Lappe and Heaney, 2008; Lappe *et al.*, 2007; Prince *et al.*, 2008; Trivedi *et al.*, 2003) suggest that vitamin D might protect against the development of cancer and CVD. Plausible biological mechanisms support this hypothesis (Deeb *et al.*, 2007; Krishnan and Feldman, 2011; Manson and Bassuk, 2009). As shown in Figure 35.1, vascular smooth muscle cells, endothelial cells, cardiomyocytes, and immune-system cells all produce 1α-hydroxylase – which converts 25(OH)D to $1,25(OH)_2D$, the natural ligand of the vitamin D receptor – or express the vitamin D receptor. $1,25(OH)_2D$ appears to inhibit vascular smooth muscle cell proliferation and vascular calcification, control volume homeostasis and blood pressure via regulation of the renin-angiotensin-aldosterone system, exert anti-inflammatory effects, and improve insulin sensitivity and secretion (Bassuk and Manson, 2009). Through binding to VDR, $1,25(OH)_2D$ has also been shown to promote cell differentiation, inhibit cancer-cell proliferation, and exhibit proapototic and antiangiogenic effects (Manson *et al.*, 2011). The Women's Health Initiative calcium-vitamin D trial, conducted among more than 36,000 postmenopausal participants, found that women randomized to daily calcium (1000 mg) plus low-dose vitamin D3 (400 IU) for a mean of 7 years did not have a lower risk for developing cancer or CVD compared to women randomized to placebo (Jackson *et al.*, 2006; Wactawski-Wende *et al.*, 2006), but the intervention's effect on 25(OH)D blood levels was small (Giovannucci *et al.*, 2006). However, large randomized trials of supplemental vitamin D at doses expected to produce biologically significant changes in 25(OH)D levels or designed to assess cancer or CVD events as primary prespecified endpoints are lacking. Given that vitamin D

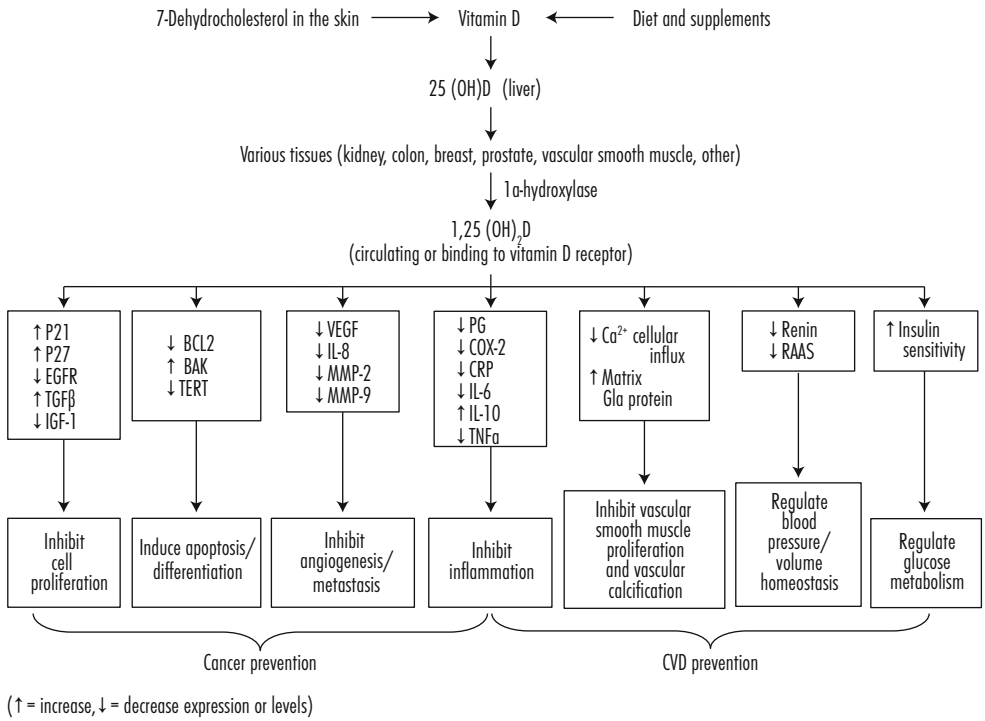

Figure 35.1. Mechanisms by which vitamin D may lower cancer and cardiovascular risk. *BAK*, BCL2-antagonist/killer; *BCL2*, B-cell chronic lymphocytic leukemia/lymphoma 2; *COX-2*, cyclooxygenase-2; *CRP*, C-reactive protein; *EGFR*, epidermal growth factor receptor; *IGF-1*, insulin-like growth factor-1; *IL-6*, interleukin-6; *IL-8*, interleukin-8; *IL-10*, interleukin-10; *MMP-2*, matrix metalloproteinase-2; *MMP-9*, matrix metalloproteinase-9; *PG*, prostaglandin; *RAAS*, renin-angiotensin-aldosterone system; *TERT*, telomerase reverse transcriptase; *TGFβ*, transforming growth factor-β; *TNFa*, tumor necrosis factor-a; *VEGF*, vascular endothelial growth factor. Reprinted from Manson *et al.* (2012), with permission from Elsevier.

insufficiency is common in the United States (e.g. a national study found that ~30% of US adults aged ≥20 had 25(OH)D levels <50 nmol/l (<20 ng/ml) and >70% had 25(OH)D levels <80 nmol/l (<32 ng/ml) (Yetley, 2008)), clarifying the possible role of vitamin D supplementation for cancer and CVD prevention is of critical public health importance.

35.2 Methods

35.2.1 Overview

To test whether supplemental vitamin D and another promising nutritional intervention – the marine omega-3 fatty acids EPA and DHA, which are found in fish and fish oil – are effective for the primary prevention of cancer and CVD, our research group is conducting the *VIT*amin D and

Omeg*A*-3 Tria*L* (VITAL), a randomized, double-blind, placebo-controlled clinical trial among 20,000 US men aged ≥50 and women aged ≥55 without a history of cancer or CVD at baseline. African Americans (blacks) are being oversampled. In a 2×2 factorial design, participants are being randomly assigned to a mean of 5 years of treatment with vitamin D3 (cholecalciferol; 2,000 IU/day) and marine omega-3 fatty acids (Omacor® fish oil, EPA + DHA, 1 g/day) supplements (or placebos). The 2×2 factorial design permits a cost-effective assessment of the independent (as well as synergistic) effects of the interventions. With the exception of some ancillary studies and the in-clinic visit component in a subcohort, the trial is being conducted by mail, which also adds to its cost effectiveness. Baseline blood samples will be collected in at least 80% of participants (n=16,000), with follow-up samples collected in ~6,000 participants. Annual questionnaires assess compliance with treatment (plasma biomarker measures will also assess compliance in a random sample of participants), use of non-study supplements, occurrence of endpoints, and risk factors for cancer and CVD. Self-reported endpoints are confirmed by medical record review by study physicians blinded to treatment assignment, and deaths are ascertained through national registries and other sources. Figure 35.2 provides an overview of the study design. Because this is a textbook on vitamin D, we focus on the vitamin D component of the trial. We refer readers interested in the omega-3 fatty acid component of the trial to our recent design paper (Manson *et al.*, 2012), from which this chapter has been adapted.

VITAL and its ancillary studies are funded by the US National Institutes of Health. The study pills and placebos are being donated by Pharmavite LLC of Northridge, California (vitamin D3) and Pronova BioPharma of Norway (Omacor® fish oil). (Both study pills are packaged together in monthly calendar packs [2 pills per day] to assist with compliance in pill taking; packaging is done at GPSI, Inc., in New York, with support from Pronova.) VITAL has received approval from the Institutional Review Board of Partners Healthcare/Brigham and Women's Hospital.

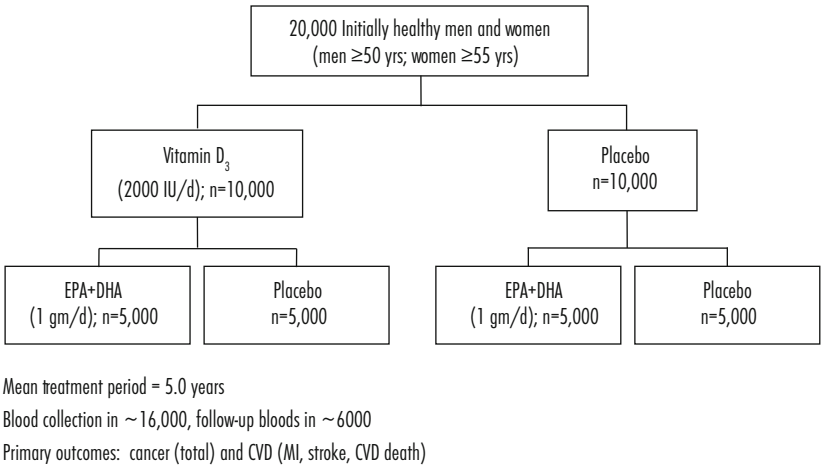

Figure 35.2. The *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL) design. Reprinted from Manson *et al.* (2012), with permission from Elsevier.

The study agents have received Investigational New Drug Approval from the US Food and Drug Administration. An independent Data and Safety Monitoring Board meets yearly to review the trial's progress and the unblinded data on endpoints and possible adverse effects in order to advise continuation, modification, or early termination of the trial. VITAL is registered at clinicaltrials.gov (NCT01169259), and the study website is www.vitalstudy.org.

35.2.2 Study aims

The primary aims of VITAL are to test whether supplemental vitamin D and/or marine omega-3 fatty acids lower the risk for total cancer and major CVD events, a composite endpoint of MI, stroke, and cardiovascular mortality. The secondary aims are to test whether each study agent reduces the risk for site-specific cancers, including incident colorectal cancer, breast cancer (in women), and prostate cancer (in men); total cancer mortality; an expanded composite cardiovascular endpoint of MI, stroke, cardiovascular mortality, and coronary revascularization (CABG or PCI); and the individual components of the primary cardiovascular endpoint. The tertiary aims are to explore whether the study agents have synergistic or additive effects on the risk for total cancer, major CVD events, and the secondary endpoints, and to explore whether the effect of the study agents on cancer and CVD risk varies by baseline blood levels of these nutrients, and – for vitamin D – by race and body mass index. Studying the effect of supplemental vitamin D in blacks is critical because this group is at higher risk for vitamin D deficiency than whites – dark skin synthesizes less vitamin D in response to solar radiation and US blacks tend to have lower intakes of dietary and supplemental vitamin D (Harris, 2006; Moore *et al.*, 2005) – and also at higher risk for certain cardiovascular events (e.g. stroke) (Harris, 2011) and cancers (e.g. prostate cancer) (American Cancer Society, 2009), as well as mortality from CVD (Harris, 2011) and cancer (American Cancer Society, 2009). Similarly, studying the effect of vitamin D supplementation in people who are obese is important, as the prevalence of obesity in the US is high (Flegal *et al.*, 2012) and obese individuals are at elevated risk for low vitamin D status (in part because of decreased bioavailability of this fat-soluble vitamin (Harris and Dawson-Hughes, 2007; Martins *et al.*, 2007)) as well as for CVD and certain cancers (Bassuk and Manson, 2008).

35.2.3 Vitamin D intervention

Our review of the literature suggests that testing a vitamin D dose of 2,000 IU/day best balances efficacy and safety concerns. With respect to efficacy, we aim to achieve a sufficient difference in vitamin D status between the treatment and placebo groups to detect reductions in the primary endpoints of cancer and CVD. In 2008, when VITAL was designed, the RDAs were 400 IU/day for US adults aged 50-70 and 600 IU/day for adults aged >70 (Institute of Medicine Food and Nutrition Board, 1999). In 2011, these RDAs increased to 600 IU/day and 800 IU/day, respectively (Institute of Medicine, 2011). These RDAs correspond to a serum 25(OH)D level of 50 nmol/l and are sufficient to maintain bone health in ≥97.5% of the US and Canadian population (Institute of Medicine, 2011). Although the evidence is not conclusive, some data suggest that higher vitamin D intakes may yield additional health benefits. For example, in a review of studies of serum 25(OH)D in relation to various outcomes, including colorectal cancer, falls, fractures,

physical functioning, and dental health, Bischoff-Ferrari *et al.* (2006) found that advantageous 25(OH)D levels began at 75 nmol/l, and optimal levels were between 90-100 nmol/l. An older individual generally requires an oral vitamin D intake of at least 800-1000 IU/day to achieve a serum 25(OH)D of 75 nmol/l (Dawson-Hughes *et al.*, 2005). Among the Women's Health Initiative cohort (a population similar to that of VITAL), 400 IU/day of vitamin D was estimated to have raised median plasma 25(OH)D only modestly – from 42.3 to 54.1 nmol/l (Giovannucci *et al.*, 2006; Wactawski-Wende *et al.*, 2006). A study by Aloia *et al.* (2008) found a nonlinear dose-response relation between serum 25(OH)D and vitamin D intake; the rate of increase in 25(OH)D level was less at higher levels of intake. Taken together, the latter two findings suggest that 2,000 IU/day of vitamin D would be required to reach the postulated optimal level of 90-100 nmol/l in participants assigned to active vitamin D. Given this dose, the expected difference in achieved 25(OH)D levels between the active treatment and placebo groups is ~50 nmol/l.

With respect to safety, because (a) individuals who report supplemental vitamin D intakes of more than 800 IU/day are not permitted to enroll in VITAL and (b) average dietary vitamin D intake in the US is ~200 IU/day (Harris, 2007), few if any participants randomized to active vitamin D will be consuming a total vitamin D dose of more than 3,000 IU/day, which is below the safety limit of 4,000 IU/day set by US and European authorities (Hathcock *et al.*, 2007; Institute of Medicine, 2011). Moreover, because nonstudy intakes of vitamin D at RDA levels are allowed, participants randomized to placebo are not expected to develop deficiency. To minimize risk for potential side effects of supplemental vitamin D, which include gastrointestinal symptoms, hypercalcemia, and kidney stones, participants are asked to limit calcium intake to 1,200 mg/day from all supplemental sources, and persons with a history of hypercalcemia or sarcoidosis (or other chronic granulomatous diseases) are not permitted to enroll in the trial. A random subsample of participants will be monitored for kidney dysfunction and abnormal blood levels of calcium and parathyroid hormone.

We decided against including calcium as a component of the intervention, for several reasons. First, a separate assessment of the effects of vitamin D, calcium, and calcium-plus-vitamin D would entail a much larger and more expensive trial than one of vitamin D alone. Second, the widespread use of supplemental calcium by older women would greatly reduce the pool of eligible females. Third, calcium plus vitamin D was associated with a statistically significant 17% increase in kidney stone risk in the Women's Health Initiative (Jackson *et al.*, 2006). Fourth, calcium supplements may more quickly increase blood calcium levels than dietary calcium, which could lead to greater calcium deposition in coronary arteries and an increased risk for coronary heart disease (Reid and Bolland, 2012), although this remains unproven (Wang *et al.*, 2012). Fifth, high calcium or milk intakes have been associated with an increased prostate cancer risk in some studies (Ahn *et al.*, 2007; Park *et al.*, 2007; Stacewicz-Sapuntzakis *et al.*, 2008).

35.2.4 Study population

The randomized study population will consist of 20,000 apparently healthy adults, half of whom are men aged ≥50 and half of whom are women aged ≥55. These are ages at which chronic disease

rates start rising substantially. Recruitment for the study is currently occurring throughout the United States, and blacks are being oversampled (the goal is a study population that is 25% black). Because this is a primary prevention trial, eligible individuals cannot have a history of cancer (except non-melanoma skin cancer), MI, stroke, transient ischemic attack, angina pectoris, or coronary revascularization (CABG or PCI). In addition, they must agree to limit consumption of supplemental vitamin D to 800 IU/day or less and supplemental calcium to 1,200 mg/day or less from all supplemental sources combined and also avoid taking fish oil supplements while participating in the trial. For safety reasons, individuals with the following conditions cannot participate: renal failure or dialysis, hypercalcemia, hypo- or hyperparathyroidism, severe liver disease (cirrhosis), or sarcoidosis or other granulomatous diseases such as chronic active tuberculosis or Wegener's granulomatosis; allergy to soy (an ingredient in the vitamin D placebo pill) or fish/shellfish (for the marine omega-3 fatty acid intervention); or other serious illness that would preclude participation. Individuals who satisfy these requirements, sign the informed consent form, and demonstrate good compliance in pill taking – defined as taking ≥2/3 of the study pills during the run-in period – are eligible for randomization.

35.2.5 Recruitment, run-in, randomization, follow-up, and endpoint determination procedures

Our primary recruitment strategy is to mail invitational letters to individuals on commercially available membership lists of professional and other organizations, subscription lists of magazines that appeal to people likely to be eligible for the trial, and lists of residents within certain geographic regions (e.g. cities with large black populations and regions within driving distance of Boston (see 'Ancillary studies and the Clinical and Translational Science Center subcohort' later in the chapter)). We are also recruiting potential participants via direct appeals in articles and advertisements in print media. We have also invited participants in one of our previously completed trials, the Women's Health Study (Ridker *et al.*, 2005), to join VITAL. Finally, we are employing targeted recruitment efforts in the black community, including neighborhood-based presentations, the creation and distribution of specialized information about the study supplements and the burden of cancer and CVD in blacks, and other initiatives, to achieve our goal of a study population that is 25% black.

Potential participants receive the following materials by postal mail: (1) an invitational letter that explains the rationale for VITAL and outlines what participation entails; (2) an informed consent form; (3) brief questionnaires containing items on demographics; medical history; allergy to fish or soy; current use of supplements containing vitamin D or fish oil; current use of other supplements or medications; dietary intake of vitamin D and consumption of fish; cancer and vascular risk factors; and (4) pre-paid envelopes for returning study forms. Questionnaire responses are evaluated to determine respondents' willingness and eligibility to enter the run-in phase of the trial. Our goal is to identify 40,000 individuals to participate in this phase.

During the run-in phase, eligible individuals take one placebo vitamin D pill and one placebo fish oil pill per day for 3 months. The run-in is helpful in selecting excellent compliers for long-

term follow-up, which increases the trial's power (Lang *et al.*, 1991). Because some effects of the interventions on the endpoints of interest (particularly cancer) may be chronic, it is not appropriate to use active agent in the run-in and then randomize to placebo. A placebo run-in also maximizes the likelihood of detecting side effects during the trial, should such side effects exist. If the active agents were used during the run-in, potential participants may drop out not only because of poor compliance but also because of side effects, and the true rate of the latter would be underestimated among those ultimately randomized. Individuals are randomized into the trial only if, during the run-in, they demonstrate good compliance in pill taking, defined as taking ≥2/3 of the study pills; report no new history of cancer or cardiovascular endpoint under study, hypercalcemia, sarcoidosis, or other serious illness; and remain willing to follow the guidelines on non-study use of supplemental vitamin D, calcium, and fish oil. We estimate that half of the 40,000 individuals who enter the run-in (n=20,000) will be randomized to a mean of 5 years of treatment with vitamin D and/or marine omega-3 fatty acid supplements (or their placebos).

Randomized participants receive follow-up questionnaires at six months and one year after randomization and annually thereafter. The questionnaires include items on compliance with randomized treatments, use of nonstudy supplemental vitamin D and marine omega-3 fatty acids, development of study endpoints and other illnesses, cancer and cardiovascular risk factors, and potential side effects of the study agents. Non-responders receive two additional requests by mail and are then telephoned to collect study data. At a minimum, vital status is ascertained. At 6-month intervals between the annual follow-ups, participants receive a short questionnaire on the development of new primary endpoints (cancer, MI and stroke), pill-taking compliance, and address changes.

Participants who report an endpoint of interest are asked to sign a medical release for relevant hospital and physician records. Using a defined protocol, study physicians blinded to the randomized treatment assignment review the records to confirm or disconfirm the endpoint. Cancer diagnoses are confirmed with histologic or cytologic data or strong clinical evidence accompanied by radiologic evidence or laboratory markers (Fritz *et al.*, 2000). MI is confirmed using Joint European Society of Cardiology/American College of Cardiology Foundation/ American Heart Association/World Heart Federation Task Force for the Redefinition of Myocardial Infarction criteria (Thygesen *et al.*, 2007). Stroke is confirmed according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (Adams *et al.*, 1993). Cardiovascular mortality is confirmed by convincing evidence of a cardiovascular event from all available sources, including death certificates, hospital records, autopsy reports, and, for deaths outside the hospital, observer accounts.

If a death is reported by a family member, copies of the medical records and death certificate are requested from the next of kin. Alternatively, a copy of the death certificate is obtained from the vital records bureau in the state where the death occurred. Study physicians review the records to assign a cause of death. If records are not available, the National Death Index Plus is searched to

obtain an International Classification of Disease-coded cause of death based on death-certificate information.

35.2.6 Blood samples

Fasting blood samples are collected at baseline (i.e. during the run-in) from as many participants as are willing to provide them. We expect that 80% of the 20,000 participants who are ultimately randomized will provide a baseline sample (n=16,000). Follow-up fasting blood samples will also be collected at years 1, 2, and/or 4 from a random subset of ~6,000 participants who provide baseline samples. The procedure for collecting the baseline sample is as follows: during the run-in, individuals are mailed a blood collection kit, including a freezer pack and overnight courier air bill, and are given the option of having their blood drawn by their own healthcare provider, at a local blood-drawing facility, or at home by a national company that provides phlebotomy services. Individuals are asked to ship the blood sample in the freezer pack to our laboratory within 24 hours of the blood draw. Upon receipt, the samples are centrifuged to separate plasma, serum, red blood cells, and buffy coat, which are stored in nitrogen freezers (-170 °C) within 30-36 hours of the blood draw. Identical procedures will be used for the follow-up blood collection.

The baseline blood samples will permit an assessment of whether treatment effects are modified by baseline blood levels of 25(OH)D (for vitamin D) and EPA+DHA (for the marine omega-3 fatty acids). The follow-up blood samples will allow an assessment of pill-taking compliance (see also next paragraph); changes in biomarkers in response to the intervention; and, in the placebo group, the effect of changing trends in background intakes of vitamin D and marine omega-3 fatty acids. In addition, changes in blood calcium and parathyroid hormone levels will be measured to assess possible hypercalcemia, a potential side effect of high vitamin D intake. The blood samples will also be used to study whether the interventions affect biomarkers related to lipids, glucose tolerance, inflammation, endothelial dysfunction, thrombosis, insulin, and insulin-like growth factor pathways. Finally, the samples will allow for future biochemical and genetic studies.

35.2.7 Assessment of compliance

The primary measure of compliance with pill-taking is participants' responses to questionnaire items on adherence. Our experience with large trials similar to VITAL indicates that although most participants make an effort to take their pills, those who fail to do so will admit this. Thus, blood levels and self-reported adherence data have been strongly correlated in our past trials (Satterfield *et al.*, 1990). However, to obtain an objective measure of compliance, we will periodically visit with limited advance notice a small group of randomly sampled local participants to draw a blood sample for determination of 25(OH)D and EPA+DHA levels. The distribution of these values will be compared between the active treatment and placebo groups, and compared with the questionnaire data on adherence, as a check on the validity of the latter. In addition, as described in the previous paragraph, follow-up blood samples collected from ~6,000 participants will allow another assessment of pill-taking compliance.

35.2.8 Assessment of background intake of vitamin D

Measuring participants' background intakes of vitamin D, marine omega-3 fatty acids, and other nutrients at baseline and during the course of the trial is necessary to determine whether the effects of the study agents depend on these variables. For example, partipants with low baseline vitamin D intakes may benefit more from the vitamin D intervention than those with high baseline intakes. At baseline, two years, and at the end of the trial, participants will be asked to answer a semi-quantitative food frequency questionnaire, which accurately categorizes an individual's intake of various nutrients, including vitamin D and marine omega-3 fatty acids (Feskanich *et al.*, 1993, 2003; Hunter *et al.*, 1992; Jacques *et al.*, 1993; Rimm *et al.*, 1992; Salvini *et al.*, 1989). Respondents estimate their average intake over the past year of various foods, beverages, and supplements that contain vitamin D, marine omega-3 fatty acids, and other nutrients. Additional questions on use of non-study supplements or drugs containing vitamin D or marine omega-3 fatty acids are asked at baseline, six months, and on yearly follow-up questionnaires. Nutrient intakes from food alone, supplements alone, and the two sources combined will be analyzed to determine whether the effects of the study agents vary according to these variables.

35.2.9 Data analysis and statistical power

The main analyses will compare the main effects of intention-to-treat with vitamin D and with marine omega-3 fatty acids on the primary cancer and CVD endpoints of interest, using a Cox proportional hazards model to allow for variable follow-up lengths (Cox, 1972).

To compute statistical power, the following assumptions were made: (1) a 2×2 factorial trial in 10,000 men aged ≥50 and 10,000 women aged ≥55 at baseline; (2) independent and equal allocation of participants to each treatment (achieved by randomization); (3) an age distribution based on that observed at baseline in our past trials for men aged ≥50 and women aged ≥55, but limited to 30% in the youngest age groups (50-59 years in men and 55-64 in women); (4) age-specific event rates based on the observed rates in the first 5 years of follow-up in our trials with similarly aged populations; (5) a target of 25% blacks, with a corresponding increase in rates of CVD (Hozawa *et al.*, 2007) and cancer (Surveillance Research Program of the National Cancer Institute); (6) a trial follow-up period of 5 years, with little loss to follow-up as achieved in our past trials; and (7) compliance (80%) similar to that in published trials upon which our estimated RR reductions are based. Power was calculated for a two-sided test with a significance level of 0.05. Assuming that only vitamin D is effective (i.e. that omega-3s have no effect), with 5 years of treatment and follow-up there will be 91% power to detect an observed RR of 0.85 for the primary endpoint of total cancer incidence and 92% power to detect an observed RR of 0.80 for the primary composite endpoint of MI, stroke, and cardiovascular mortality (99% power for the expanded CVD outcome that includes coronary revascularizations). For site-specific cancers and individual CVD outcomes, the trial will be powered to detect 25-30% reductions in risk. If the vitamin D and the omega-3 interventions interact, power will be affected to the extent of the interaction. Should the interventions act synergistically, power would increase. Should the agents interact in a subadditive fashion, power would be reduced.

35.2.10 Ancillary studies and the Clinical and Translational Science Center subcohort

VITAL is expected to shed light on the effect of vitamin D on health outcomes other than cancer and CVD through well-integrated ancillary studies. These ancillary studies will assess whether vitamin D can prevent diabetes and glucose intolerance; hypertension; cognitive decline; depression; osteoporosis and fracture; physical disability and falls; asthma and other respiratory diseases; infections; kidney disease; anemia; eye problems such as macular degeneration and dry eye disease; chronic knee pain symptoms; and autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, and thyroid diseases.

In addition, a subcohort of 1000 participants who live within driving distance of the CTSC at Brigham and Women's Hospital in Boston, Massachusetts are having a detailed health assessment at the CTSC site at or near the end of the run-in period (prior to randomization) and will have a repeat assessment two years later. The two health assessments will use the same protocol to gather basic clinical data as well as data on variables related to ancillary study aims (e.g. glucose tolerance testing, physical performance batteries, lung function exams, cognitive assessments, and structured interviews to diagnose depression). Non-invasive imaging techniques are also employed to collect data for other ancillary studies, including dual energy X-ray absorptiometry to measure bone density and body composition; mammography to assess breast tissue density; and Doppler echocardiography to assess left ventricular function. The results of the latter two investigations may help to clarify mechanisms by which vitamin D possibly protects against breast cancer and CVD, respectively.

At the CTSC visits, blood samples are drawn not only for glucose tolerance testing but also for assays of 25(OH)D and EPA+DHA levels. The timing of the second visit will be matched by month to the first visit to minimize variability in seasonal sun exposure, a major contributor to within-person variation in 25(OH)D. The CTSC visits represent an important opportunity for in-person contact with some participants, allowing for in-depth phenotyping and face-to-face validation of the remote assessment methods used in the main trial and ancillary studies. For example, in-person cognitive testing at the CTSC visit will be used to validate telephone-based assessments in the cognitive function ancillary study, and in-person structured diagnostic interviews for clinical depression at the CTSC visit will be used to validate clinical depression cases identified by screening checklists in the depression ancillary study.

The CTSC subcohort is expected to mirror the diverse racial/ethnic composition of the overall VITAL study population. Members of the subcohort are being randomized equally into the four treatment groups created by the factorial design (i.e. 250 participants per treatment group).

35.2.11 Strengths and limitations

Among the many strengths of VITAL is the fact that it is testing two promising nutritional agents (vitamin D and marine omega-3 fatty acids) for the prevention of two major diseases (cancer and CVD) in a racially/ethnically diverse population. The trial is using a mail-based, large simple trial

design, at great cost efficiency. The study includes the collection and storage of baseline blood samples in most of the cohort to allow assessment of effect modification by baseline 25(OH)D and EPA+DHA levels, and the collection and storage of follow-up samples in a large subgroup of participants to allow assessment of pill-taking compliance; changes in biomarkers in response to treatment; and, in the placebo group, the effect of changing background intakes of vitamin D and marine omega-3 fatty acids. The trial has excellent power to detect small to moderate effects of the study agents on the primary endpoints of interest and will also advance our knowledge of the agents' effects on other health outcomes through well-integrated ancillary studies. Among the study's limitations is the fact that it is examining only a single dose of each agent, precluding an assessment of potential dose-response relationships. However, the chosen dose was selected after an extensive review of available data, with careful consideration given to both efficacy and safety. Latent effects, especially for cancer endpoints, may produce artifactually null results. However, accumulating data suggest that vitamin D may act at later stages of carcinogenesis (Deeb *et al.*, 2007; Lappe *et al.*, 2007; Welsh, 2007), suggesting that salutary effects of treatment could emerge within 5 years. Finally, because the study population is older, the findings may not apply to younger individuals. However, older populations have higher rates of disease, allowing the trial to be completed in a timely fashion, which is important for cost efficiency.

35.3 Conclusion

There has been much recent interest among both medical professionals and the lay public about possible nonskeletal health benefits of vitamin D. Many healthcare providers now routinely include vitamin D blood tests as part of lab work and recommend vitamin D supplements to patients. Sales of such supplements have dramatically increased in recent years (Parker-Pope, 2010). However, the evidence to support such enthusiasm for vitamin D is far from compelling. In a 2011 report, the Institute of Medicine comprehensively reviewed the literature on this vitamin in relation to multiple health outcomes and concluded that although there is strong evidence that vitamin D – at doses of 600 to 800 IU/day – confers bone benefits, available data are insufficient to determine whether higher vitamin D protects against the development of nonskeletal diseases, including cancer and CVD (Institute of Medicine, 2011; Ross *et al.*, 2011). To address this knowledge gap, the Imstitute of Medicine called for additional studies, including large randomized controlled trials such as VITAL, to determine whether high-dose vitamin D supplements confer nonskeletal benefits and whether such supplements pose any health risks. Randomized trials of other single-agent nutritional interventions, including certain antioxidant vitamins, selenium, B-vitamins, and calcium, have disproved some purported health benefits for these supplements and even uncovered harmful effects that may have otherwise gone unrecognized (Bjelakovic *et al.*, 2007; Bolland *et al.*, 2010; Byers, 2010; Kris-Etherton *et al.*, 2004; Lee *et al.*, 2005). Indeed, some data suggest adverse effects on CVD (Freedman *et al.*, 2007; Wang *et al.*, 2008), pancreatic cancer (Stolzenberg-Solomon *et al.*, 2009), all-cause mortality (Melamed *et al.*, 2008), and even fracture (Sanders *et al.*, 2010) at very high 25(OH)D levels. The results of VITAL are expected to shape clinical and public health guidelines regarding the use of supplemental vitamin D for the primary prevention of cancer, CVD, and other health conditions.

Acknowledgments

VITAL is supported by grant U01 CA138962, which includes support from the National Cancer Institute; National Heart, Lung and Blood Institute; Office of Dietary Supplements; National Institute of Neurological Disorders and Stroke; and the National Center for Complementary and Alternative Medicine. The ancillary studies are supported by grants from several other NIH institutes. Members of the Data and Safety Monitoring Board include Lawrence S. Cohen, Theodore Colton, Mark A. Espeland, I. Craig Henderson, Alice H. Lichtenstein, Rebecca A. Silliman, and Nanette K. Wenger (chair), and Josephine Boyington, Cindy D. Davis, Rebecca B. Costello, Lawrence Fine, and Peter Greenwald (ex-officio members).

References

Abbas, S., Linseisen, J., Slanger, T., Kropp, S., Mutschelknauss, E.J., Flesch-Janys, D. and Chang-Claude, J., 2008. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer – results of a large case-control study. Carcinogenesis 29, 93-99.

Adams Jr., H.P., Bendixen, B.H., Kappelle, L.J., Biller, J., Love, B.B., Gordon, D.L. and Marsh, E.E., 3rd, 1993. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 24, 35-41.

Ahn, J., Albanes, D., Peters, U., Schatzkin, A., Lim, U., Freedman, M., Chatterjee, N., Andriole, G.L., Leitzmann, M.F. and Hayes, R.B., 2007. Dairy products, calcium intake, and risk of prostate cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiology, Biomarkers & Prevention 16, 2623-2630.

Aloia, J.F., Patel, M., Dimaano, R., Li-Ng, M., Talwar, S.A., Mikhail, M., Pollack, S. and Yeh, J.K., 2008. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. American Journal of Clinical Nutrition 87, 1952-1958.

American Cancer Society, 2009. Cancer Facts & Figures for African Americans 2009-2010. American Cancer Society, Atlanta.

Anderson, J.L., May, H.T., Horne, B.D., Bair, T.L., Hall, N.L., Carlquist, J.F., Lappe, D.L. and Muhlestein, J.B., 2010. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. American Journal of Cardiology 106, 963-968.

Bassuk, S.S. and Manson, J.E., 2008. Health consequences of obesity/overweight. In: H.K. Heggenhougen and S. Quah (eds.), International Encyclopedia of Public Health, Volume 4. Academic Press, San Diego, pp. 589-602.

Bassuk, S.S. and Manson, J.E., 2009. Does vitamin D protect against cardiovascular disease? Journal of Cardiovascular Translational Research 2, 245-250.

Bertone-Johnson, E.R., Chen, W.Y., Holick, M.F., Hollis, B.W., Colditz, G.A., Willett, W.C. and Hankinson, S.E., 2005. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. Cancer Epidemiology, Biomarkers & Prevention 14, 1991-1997.

Bischoff-Ferrari, H.A., Giovannucci, E., Willett, W.C., Dietrich, T. and Dawson-Hughes, B., 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. American Journal of Clinical Nutrition 84, 18-28.

Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.G. and Gluud, C., 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297, 842-857.

Bolland, M.J., Avenell, A., Baron, J.A., Grey, A., MacLennan, G.S., Gamble, G.D. and Reid, I.R., 2010. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ 341, c3691.

Byers, T., 2010. Anticancer vitamins du jour – the ABCED's so far. American Journal of Epidemiology 172, 1-3.

Cox, D.R., 1972. Regression models and life-tables. Journal of the Royal Statistical Society Series B 34, 187-220.

Dawson-Hughes, B., Heaney, R.P., Holick, M.F., Lips, P., Meunier, P.J. and Vieth, R., 2005. Estimates of optimal vitamin D status. Osteoporosis International 16, 713-716.

Deeb, K.K., Trump, D.L. and Johnson, C.S., 2007. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nature Reviews: Cancer 7, 684-700.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., Kinkeldei, J., Boehm, B.O., Weihrauch, G. and Maerz, W., 2008. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Archives of Internal Medicine 168, 1340-1349.

Feskanich, D., Rimm, E.B., Giovannucci, E.L., Colditz, G.A., Stampfer, M.J., Litin, L.B. and Willett, W.C., 1993. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. Journal of the American Dietetic Association 93, 790-796.

Feskanich, D., Willett, W.C. and Colditz, G.A., 2003. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. American Journal of Clinical Nutrition 77, 504-511.

Flegal, K.M., Carroll, M.D., Kit, B.K. and Ogden, C.L., 2012. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 307, 491-497.

Freedman, D.M., Looker, A.C., Chang, S.C. and Graubard, B.I., 2007. Prospective study of serum vitamin D and cancer mortality in the United States. Journal of the National Cancer Institute 99, 1594-1602.

Fritz, A.G., Percy, C., Jack, A., Shanmugaratham, K., Sobin, L.H., Parkin, M.D. and Whelan, S., 2000. International Classification of Diseases for Oncology (ICD-O), Third Edition. World Health Organization, Geneva.

Giovannucci, E., Liu, Y., Hollis, B.W. and Rimm, E.B., 2008. A prospective study of 25-hydroxyvitamin D and risk of myocardial infarction in men. Archives of Internal Medicine 168, 1174-1180.

Giovannucci, E., Liu, Y., Rimm, E.B., Hollis, B.W., Fuchs, C.S., Stampfer, M.J. and Willett, W.C., 2006. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. Journal of the National Cancer Institute 98, 451-459.

Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M., Newmark, H.L., Giovannucci, E., Wei, M. and Holick, M.F., 2005. Vitamin D and prevention of colorectal cancer. Journal of Steroid Biochemistry and Molecular Biology 97, 179-194.

Gorham, E.D., Garland, C.F., Garland, F.C., Grant, W.B., Mohr, S.B., Lipkin, M., Newmark, H.L., Giovannucci, E., Wei, M. and Holick, M.F., 2007. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta-analysis. American Journal of Preventive Medicine 32, 210-216.

Grant, W.B., 2002. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer 94, 1867-1875.

Grant, W.B., 2003. Ecologic studies of solar UV-B radiation and cancer mortality rates. Recent Results in Cancer Research 164, 371-377.

Harris, S.S., 2006. Vitamin D and African Americans. Journal of Nutrition 136, 1126-1129.

Harris, S.S., 2011. Does vitamin D deficiency contribute to increased rates of cardiovascular disease and type 2 diabetes in African Americans? American Journal of Clinical Nutrition 93(5), 1175S-1178S.

Harris, S.S. and Dawson-Hughes, B., 2007. Reduced sun exposure does not explain the inverse association of 25-hydroxyvitamin D with percent body fat in older adults. Journal of Clinical Endocrinology and Metabolism 92, 3155-3157.

Harris, W.S., 2007. International recommendations for consumption of long-chain omega-3 fatty acids. Journal of Cardiovascular Medicine (Hagerstown, Md.) 8 Suppl 1, S50-52.

Hathcock, J.N., Shao, A., Vieth, R. and Heaney, R., 2007. Risk assessment for vitamin D. American Journal of Clinical Nutrition 85, 6-18.

Hozawa, A., Folsom, A.R., Sharrett, A.R. and Chambless, L.E., 2007. Absolute and attributable risks of cardiovascular disease incidence in relation to optimal and borderline risk factors: comparison of African American with white subjects – atherosclerosis risk in communities study. Archives of Internal Medicine 167, 573-579.

Hunter, D.J., Rimm, E.B., Sacks, F.M., Stampfer, M.J., Colditz, G.A., Litin, L.B. and Willett, W.C., 1992. Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. American Journal of Epidemiology 135, 418-427.

Institute of Medicine, 2011. Dietary reference intakes for calcium and vitamin D. The National Academies Press, Washington DC.

Institute of Medicine Food and Nutrition Board, 1999. Dietary reference intakes: calcium, phosphorous, magnesium, vitamin D and fluoride. National Academies Press, Washington DC.

Jackson, R.D., LaCroix, A.Z., Gass, M., Wallace, R.B., Robbins, J., Lewis, C.E., Bassford, T., Beresford, S.A., Black, H.R., Blanchette, P., Bonds, D.E., Brunner, R.L., Brzyski, R.G., Caan, B., Cauley, J.A., Chlebowski, R.T., Cummings, S.R., Granek, I., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Johnson, K.C., Judd, H., Kotchen, J.M., Kuller, L.H., Langer, R.D., Lasser, N.L., Limacher, M.C., Ludlam, S., Manson, J.E., Margolis, K.L., McGowan, J., Ockene, J.K., O'Sullivan, M.J., Phillips, L., Prentice, R.L., Sarto, G.E., Stefanick, M.L., Van Horn, L., Wactawski-Wende, J., Whitlock, E., Anderson, G.L., Assaf, A.R. and Barad, D., 2006. Calcium plus vitamin D supplementation and the risk of fractures. New England Journal of Medicine 354, 669-683.

Jacques, P.F., Sulsky, S.I., Sadowski, J.A., Phillips, J.C., Rush, D. and Willett, W.C., 1993. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. American Journal of Clinical Nutrition 57, 182-189.

Kris-Etherton, P.M., Lichtenstein, A.H., Howard, B.V., Steinberg, D. and Witztum, J.L., 2004. Antioxidant vitamin supplements and cardiovascular disease. Circulation 110, 637-641.

Krishnan, A.V. and Feldman, D., 2011. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. Annual Review of Pharmacology and Toxicology 51, 311-336.

Lang, J.M., Buring, J.E., Rosner, B., Cook, N. and Hennekens, C.H., 1991. Estimating the effect of the run-in on the power of the Physicians' Health Study. Statistics in Medicine 10, 1585-1593.

Lappe, J.M. and Heaney, R.P., 2008. Calcium supplementation: Results may not be generalisable. BMJ 336, 403.

Lappe, J.M., Travers-Gustafson, D., Davies, K.M., Recker, R.R. and Heaney, R.P., 2007. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. American Journal of Clinical Nutrition 85, 1586-1591.

Lee, I.M., Cook, N.R., Gaziano, J.M., Gordon, D., Ridker, P.M., Manson, J.E., Hennekens, C.H. and Buring, J.E., 2005. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA 294, 56-65.

Lowe, L.C., Guy, M., Mansi, J.L., Peckitt, C., Bliss, J., Wilson, R.G. and Colston, K.W., 2005. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. European Journal of Cancer 41, 1164-1169.

Manson, J.E. and Bassuk, S.S., 2009. Vitamin D and cardiovascular disease. Menopause Management 18, 28-31.

Manson, J.E., Bassuk, S.S., Lee, I.-M., Cook, N.R., Albert, M.A., Gordon, D., Zaharris, E., MacFadyen, J.G., Danielson, E., Lin, J., Zhang, S.M. and Buring, J.E., 2012. The *VIT*amin D and Omeg*A*-3 Tria*L* (VITAL): Rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. Contemporary Clinical Trials 33, 159-171.

Manson, J.E., Mayne, S.T. and Clinton, S.K., 2011. Vitamin D and prevention of cancer – ready for prime time? New England Journal of Medicine 364, 1385-1387.

Martins, D., Wolf, M., Pan, D., Zadshir, A., Tareen, N., Thadhani, R., Felsenfeld, A., Levine, B., Mehrotra, R. and Norris, K., 2007. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Archives of Internal Medicine 167, 1159-1165.

Melamed, M.L., Michos, E.D., Post, W. and Astor, B., 2008. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Archives of Internal Medicine 168, 1629-1637.

Mizoue, T., 2004. Ecological study of solar radiation and cancer mortality in Japan. Health Physics 87, 532-538.

Moore, C.E., Murphy, M.M. and Holick, M.F., 2005. Vitamin D intakes by children and adults in the United States differ among ethnic groups. Journal of Nutrition 135, 2478-2485.

Park, Y., Mitrou, P.N., Kipnis, V., Hollenbeck, A., Schatzkin, A. and Leitzmann, M.F., 2007. Calcium, dairy foods, and risk of incident and fatal prostate cancer: the NIH-AARP Diet and Health Study. American Journal of Epidemiology 166, 1270-1279.

Parker-Pope, T., 2010. The miracle of vitamin D: sound science, or hype? Well blog of the New York Times. Feb 1, 2010. Available at: http://well.blogs.nytimes.com/2010/02/01/the-miracle-of-vitamin-d-sound-science-or-hype/.

Pilz, S., Dobnig, H., Winklhofer-Roob, B., Riedmuller, G., Fischer, J.E., Seelhorst, U., Wellnitz, B., Boehm, B.O. and Marz, W., 2008. Low serum levels of 25-hydroxyvitamin D predict fatal cancer in patients referred to coronary angiography. Cancer Epidemiology, Biomarkers & Prevention 17, 1228-1233.

Prince, R.L., Austin, N., Devine, A., Dick, I.M., Bruce, D. and Zhu, K., 2008. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. Archives of Internal Medicine 168, 103-108.

Reid, I.R. and Bolland, M.J., 2012. Calcium supplements: bad for the heart? Heart 98, 895-896.

Ridker, P.M., Cook, N.R., Lee, I.M., Gordon, D., Gaziano, J.M., Manson, J.E., Hennekens, C.H. and Buring, J.E., 2005. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. New England Journal of Medicine 352, 1293-1304.

Rimm, E.B., Giovannucci, E.L., Stampfer, M.J., Colditz, G.A., Litin, L.B. and Willett, W.C., 1992. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. American Journal of Epidemiology 135, 1114-1126.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J. and Shapses, S.A., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. Journal of Clinical Endocrinology and Metabolism 96, 53-58.

Salvini, S., Hunter, D.J., Sampson, L., Stampfer, M.J., Colditz, G.A., Rosner, B. and Willett, W.C., 1989. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. International Journal of Epidemiology 18, 858-867.

Sanders, K.M., Stuart, A.L., Williamson, E.J., Simpson, J.A., Kotowicz, M.A., Young, D. and Nicholson, G.C., 2010. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 303, 1815-1822.

Satterfield, S., Greco, P.J., Goldhaber, S.Z., Stampfer, M.J., Swartz, S.L., Stein, E.A., Kaplan, L. and Hennekens, C.H., 1990. Biochemical markers of compliance in the Physicians' Health Study. American Journal of Preventive Medicine 6, 290-294.

Shapses, S.A. and Manson, J.E., 2011. Vitamin D and prevention of cardiovascular disease and diabetes: why the evidence falls short. JAMA 305, 2565-2566.

Stacewicz-Sapuntzakis, M., Borthakur, G., Burns, J.L. and Bowen, P.E., 2008. Correlations of dietary patterns with prostate health. Molecular Nutrition & Food Research 52, 114-130.

Stolzenberg-Solomon, R.Z., Hayes, R.B., Horst, R.L., Anderson, K.E., Hollis, B.W. and Silverman, D.T., 2009. Serum vitamin D and risk of pancreatic cancer in the Prostate, Lung, Colorectal, and Ovarian Screening Trial. Cancer Research 69, 1439-1447.

Surveillance Research Program of the National Cancer Institute, Fast Stats: An Interactive Tool for Access to SEER Cancer Statistics. Available at: http://seer.cancer.gov/faststats.

Thygesen, K., Alpert, J.S., White, H.D. and Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction, 2007. Universal definition of myocardial infarction. Circulation 116, 2634-2653.

Trivedi, D.P., Doll, R. and Khaw, K.T., 2003. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 326, 469.

Wactawski-Wende, J., Kotchen, J.M., Anderson, G.L., Assaf, A.R., Brunner, R.L., O'Sullivan, M.J., Margolis, K.L., Ockene, J.K., Phillips, L., Pottern, L., Prentice, R.L., Robbins, J., Rohan, T.E., Sarto, G.E., Sharma, S., Stefanick, M.L., Van Horn, L., Wallace, R.B., Whitlock, E., Bassford, T., Beresford, S.A., Black, H.R., Bonds, D.E., Brzyski, R.G., Caan, B., Chlebowski, R.T., Cochrane, B., Garland, C., Gass, M., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Jackson, R.D., Johnson, K.C., Judd, H., Kooperberg, C.L., Kuller, L.H., LaCroix, A.Z., Lane, D.S., Langer, R.D., Lasser, N.L., Lewis, C.E., Limacher, M.C. and Manson, J.E., 2006. Calcium plus vitamin D supplementation and the risk of colorectal cancer. New England Journal of Medicine 354, 684-696.

Wang, L., Manson, J.E. and Sesso, H.D., 2012. Calcium intake and risk of cardiovascular disease: a review of prospective studies and randomized clinical trials. American Journal of Cardiovascular Drugs 12, 105-116.

Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M. and Vasan, R.S., 2008. Vitamin D deficiency and risk of cardiovascular disease. Circulation 117, 503-511.

Welsh, J., 2007. Vitamin D and prevention of breast cancer. Acta Pharmacologica Sinica 28, 1373-1382.

Yetley, E.A., 2008. Assessing the vitamin D status of the US population. American Journal of Clinical Nutrition 88, 558S-564S.

Zittermann, A., Schleithoff, S.S. and Koerfer, R., 2005. Putting cardiovascular disease and vitamin D insufficiency into perspective. British Journal of Nutrition 94, 483-492.

Index

A

R.R. Watson (ed.), *Handbook of vitamin D in human health: Prevention, treatment and toxicity*, Human Health Handbooks no. 4, DOI 10.3920/978-90-8686-765-3, © Wageningen Academic Publishers 2013

B

C

D

E

F

G

H

N

R

S

T

W

About the edtior

Ronald Ross Watson, PhD, attended the University of Idaho but graduated from Brigham Young University in Provo, Utah, with a degree in chemistry in 1966. He earned his PhD in biochemistry from Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health, where he gained 2 years of postdoctoral research experience in immunology and nutrition.

From 1973 to 1974 Dr. Watson was assistant professor of immunology and performed research at the University of Mississippi Medical Center in Jackson. He was assistant professor of microbiology and immunology at the Indiana University Medical School from 1974 to 1978 and associate professor at Purdue University in the Department of Food and Nutrition from 1978 to 1982. In 1982 Dr. Watson joined the faculty at the University of Arizona Health Sciences Center in the Department of Family and Community Medicine of the School of Medicine. He is currently professor of health promotion sciences in the Mel and Enid Zuckerman Arizona College of Public Health.

Dr. Watson is a member of several national and international nutrition, immunology, cancer, and alcoholism research societies. Among his patents he has one on a dietary supplement; passion fruit peel extract, with more pending. He continues to do research in animals and in clinical trials on dietary supplements and health. For 30 years he was funded by the Wallace Research Foundation to study dietary supplements in health promotion. Dr. Watson has edited more than 100 books on nutrition, dietary supplements and over-the-counter agents, and drugs of abuse as scientific reference books. He has published more than 500 research and review articles.

Acknowledgments

The work of Dr. Watson's editorial assistant, Bethany L. Stevens and Wageningen's project manager, Mike Jacobs in communicating with authors and working on the manuscripts was critical to the successful completion of the book. It is very much appreciated. Support for Ms. Stevens' and Dr. Watson's work was graciously provided by the Natural Health Research Institute (www.naturalhealthresearch.org). It is an independent, non-profit organization that supports science-based research on natural health and wellness, and which is committed to informing about scientific evidence on the usefulness and cost-effectiveness of diet, supplements and a healthy lifestyle to improve health and wellness, and reduce disease. Finally the work of librarians of the Arizona Health Science Library, Mari Stoddard and Nguyen Nga Thi, was vital and very helpful in identifying key researchers who participated in the book.