ILKKA LAAKSI

Vitamin D Insufficiency in Young Finnish Men

Associations with bone stress fracture and respiratory tract infections

ACADEMIC DISSERTATION
To be presented, with the permission of
the board of the School of Medicine of the University of Tampere,
for public discussion in the Jarmo Visakorpi Auditorium,
of the Arvo Building, Lääkärinkatu 1, Tampere,
on June 8th, 2012, at 12 o'clock.

UNIVERSITY OF TAMPERE

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION
University of Tampere, School of Medicine
Centre for Military Medicine, Lahti
Finland

Supervised by
Professor Timo Ylikomi
University of Tampere
Finland
Docent Harri Pihlajamäki
University of Helsinki
Finland

Reviewed by
Professor Ilari Paakkari
University of Helsinki
Finland
Professor Tomi-Pekka Tuomainen
University of Eastern Finland
Finland

Distribution
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 40 190 9800
Fax +358 3 3551 7685
taju@uta.fi
www.uta.fi/taju
http://granum.uta.fi

Cover design by
Mikko Reinikka

Acta Universitatis Tamperensis 1736
ISBN 978-951-44-8819-1 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1207
ISBN 978-951-44-8820-7 (pdf)
ISSN 1456-954X
http://acta.uta.fi

Tampereen Yliopistopaino Oy – Juvenes Print
Tampere 2012

To Leena
To Iida, Akseli and Lotta

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by Roman numerals I to IV.

I: Laaksi IT, Ruohola J-P, Ylikomi TJ, Auvinen A, Haataja RI, Pihlajamäki HK, Tuohimaa PJ
Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. Eur J Clin Nutr. 2006 Aug;60(8):1035-8.

II: Ruohola J-P[1], Laaksi IT[1], Ylikomi TJ, Haataja RI, Mattila VM, Sahi T, Tuohimaa PJ, Pihlajamäki HK
Association between serum 25OHD concentrations and bone stress fractures in Finnish young men. J Bone Miner Res. 2006 Sep;21(9):1483-8.
[1] These authors contributed equally to this paper

III: Laaksi I, Ruohola J-P, Tuohimaa P, Auvinen A, Haataja R, Pihlajamäki H, Ylikomi T
An association of serum vitamin D concentrations <40 nmol/l with acute respiratory tract infection in young Finnish men. Am J Clin Nutr. 2007 Sep;86(3):714-7.

IV: Laaksi I, Ruohola J-P, Mattila VM, Auvinen A, Ylikomi T, Pihlajamäki H
Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. J Infect Dis. 2010 Sep 1;202(5):809-14.

ABSTRACT

Vitamin D is not an actual vitamin but a secosteroid hormone produced in the skin from 7-dehydrocholesterol after exposure to sunlight's ultraviolet B radiation. Vitamin D needs to be hydroxylated twice to reach an active form that is able to regulate gene expression through binding with vitamin D receptors (VDRs) and further to vitamin-D-responsive elements (VDREs) in vitamin-D-responsive genes. The level of the major circulating form of the hormone, serum 25OHD, is used for determination of vitamin D status. Vitamin D insufficiency can be regarded as a global issue with substantial implications for health. On account of inadequate sun exposure in wintertime, vitamin D insufficiency is commonplace among all age groups in Finland. As sunlight exposure is inadequate for vitamin D production in the skin, nutrition and supplements are the main sources of vitamin D in northern countries during winter months. Upon the recommendation of the Ministry of Social Affairs and Health, vitamin D has been added to commercial milk products and margarines since February 2003 in Finland.

In the first study, we determined the effects of national policy on vitamin D fortification in young Finnish men. The study population consisted of 196 young Finnish men (18–28 yrs) whose serum 25OHD concentrations were determined with Octeia® enzyme immunoassay by IDS in January 2003 (n = 96) or in January 2004, one year after national vitamin D fortification started. We found a 50% increase in mean serum 25OHD3 concentrations after implementation of the vitamin D fortification of dairy products. In addition, the prevalence of vitamin D insufficiency (<40 nmol/l) had decreased by 50% (from 78% in January 2003 to 35% in January 2004). The study showed that national vitamin D fortification substantially improved the vitamin D status of young Finnish men. However, 35% remained vitamin D insufficient.

The most commonly known function of vitamin D is the effect on bone mineralisation. Bone stress fractures are one of the most frequently seen types of overuse injuries in athletes and military recruits. An association was recently shown between vitamin D and bone mineral content, with a correlation between low femoral bone density and stress fractures. In the second study, we measured the serum 25OHD concentration in a population sample of military recruits to determine whether vitamin D is a predisposing factor for bone stress fractures. In this prospective study, 800 healthy Finnish military recruits with a mean age of 19 years were followed up for development of stress fractures in homogenous circumstances. Serum 25OHD concentrations were measured with enzyme immunoassay at entry into military service, and the weight, height, body mass index (BMI), physical fitness score, and result of a 12-minute running test were

measured for all subjects. In all, 756 subjects had completed the study at the end of 90-day follow-up, and subjects without a fracture constituted controls. The study found 22 recruits with a stress fracture (2.9%), the incidence being 11.6 (95% CI: 6.8–16.5) per 100 person-years. In the final multivariate analysis, the statistically significant risk factor for stress fracture in conscripts was below-median serum 25OHD level (75.8 nmol/l) OR being 3.6 (95% CI: 1.1–11.1). No statistically significant associations between BMI, age, physical fitness score, 12-minute running test or smoking and bone stress fractures were found in this study population. In conclusion, a lower serum 25OHD concentration may be a generally predisposing factor for bone stress fractures.

Vitamin D has a role in innate immunity activation; the production of antimicrobial peptides following toll-like receptor (TLR) stimulation by pathogen lipopeptides is dependent on a high enough level of 25OHD. Recent evidence suggest that differences in the ability of human populations to produce vitamin D may contribute to susceptibility to microbial infection. In the third study, we explored whether an association exists between vitamin D insufficiency and acute respiratory tract infection in young Finnish men. For this prospective study, young Finnish men (n = 800) serving at a military base in Finland were enrolled. Serum 25OHD concentrations were measured in July 2002 and the subjects were followed up for six months, and the number of days of absence from duty due to respiratory infection was calculated. The mean serum 25OHD concentration was 80 nmol/l in July 2002 (n = 756). The subjects with serum 25OHD concentrations <40 nmol/l, indicating vitamin D insufficiency, had statistically significantly more days of absence from duty due to respiratory infection. Also, a statistically significant positive association between serum 25OHD concentrations and the amount of physical exercise before induction into military service was found. In addition, smoking was statistically significantly associated with lower serum 25OHD concentrations. In conclusion, the study showed that a low vitamin D level increases the risk of acute respiratory tract infections.

There is clinical evidence of an association between vitamin D insufficiency and respiratory tract infections. There is also some evidence of prevention of infections by vitamin D supplementation. In the fourth study, we determined the effect of vitamin D supplementation on the incidence of acute respiratory tract infections in young Finnish men. For this RCT, 164 healthy conscripts were enrolled. From October to March, half of them received 10 µg of vitamin D daily and half received a placebo. Smoking was adjusted for in the study's analysis. The mean serum 25OHD concentrations were 79 nmol/l in October 2005 and 72 nmol/l in March 2006 in the vitamin D group. The corresponding concentrations in the placebo group were 74 and 51 nmol/l. There was no statistically significant difference in the number of days of absence from duty (the main outcome variable) between the vitamin D and placebo group. However, the proportion of men remaining healthy throughout the six-month study period was greater in the vitamin D group (51%) than in the placebo group (36%), p = 0.045. Further, in a Cox regression analysis with adjustment for smoking, the adjusted hazard ratio (HR) for absence from duty due to a respiratory tract

infection was lower in the vitamin D group (HR 0.71; 95% CI: 0.43–1.15). The RCT showed some evidence of a preventive effect of vitamin D supplementation against respiratory tract infection. Larger randomised controlled trials are warranted to explore this preventive effect.

TIIVISTELMÄ

D-vitamiini on hormoni, jolla tiedetään olevan merkittävä vaikutus luun mineralisaatiossa, sillä se tehostaa kalsiumin imeytymistä suolistossa ja toisaalta lisää kalsiumin takaisinimeytymistä munuaisissa. Lapsilla riisitauti ja aikuisilla osteoporoosi ovat molemmat pitkään jatkuneen D-vitamiinin vajauksen seurauksia.

Suurin osa elimistön D-vitamiinista syntyy ihossa auringon UVB -säteilyn vaikutuksesta. D-vitamiini hydroksyloituu ensin maksassa kalsidioliksi (25OHD) ja sitten joko munuaisissa ja/tai kohde-elimissä kalsitrioliksi ($1,25(OH)_2D$), joka on sen hormonaalisesti aktiivisin muoto. Tumareseptoriin (VDR) sitoutunut kalsitrioli lisää tiettyjen geenien ilmentymistä elimistössä ja säätelee siten solujen toimintoja. Uusien tutkimusten mukaan D-vitamiinilla näyttää olevan laaja merkitys terveyden ylläpitämisessä.

D-vitamiinin vajaus on maailmanlaajuinen ongelma. Pohjoisen alueen maissa, joissa auringonvalo on riittämätöntä tuottamaan D-vitamiinia ihossa, vajaus on erityisen laajaa. Suomessa D-vitamiinia ei muodostu iholla lainkaan lokakuusta maaliskuuhun ja sen vajausta onkin todettu kaikissa ikäluokissa talvikuukausina. Tilanteen parantamiseksi sosiaali- ja terveysministeriö antoi ohjeistuksen maitotuotteiden D-vitaminoinnin aloittamiseksi helmikuussa 2003. Valtion ravitsemusneuvottelukunta on antanut suosituksensa D-vitaminoinnista huhtikuussa 2010.

Ensimmäisessä osatyössä tutkimme D-vitaminoinnin vaikutuksia varusmiesten D-vitamiinipitoisuuksiin. Tutkimukseen osallistui 196 suomalaista 18-28v tervettä miestä, joiden seerumin 25OHD –pitoisuudet mitattiin sekä ennen valtakunnallista maitotuotteiden D-vitaminointia (1/2003) että noin vuosi sen jälkeen (1/2004) käyttäen entsyymi-immunomenetelmää (IDS). Tammikuussa 2003 seerumin 25OHD:n keskiarvo oli 34 nmol/l ja nousi tammikuussa 2004, vuosi D-vitaminoinnin alkamisen jälkeen 50 nmol/l:iin. Samalla D-vitamiinin vajauksesta kärsivien määrä putosi 78 %:sta 35 %:iin. Tutkimus osoitti, että kansallinen maitotuotteiden D-vitaminointi paransi merkittävästi varusmiesten D-vitamiinipitoisuuksia. Silti, D –vitamiinin vajausta todettiin joka kolmannella varusmiehellä.

Rasitusmurtuma on tyypillinen luun ylikuormituksesta johtuva seuraus sekä varusmiehillä että urheilijoilla. Aiemmin D-vitamiinin vajauksen on todettu olevan yhteydessä alentuneeseen luun mineraalimäärään (BMC). Toisaalta alentunut luun mineraalitiheys (BMD) ennakoi rasitusmurtumaa. Toisessa osatyössä selvitimme varusmiesten D-vitamiinipitoisuuden yhteyttä rasitusmurtumien syntyyn. Tähän prospektiiviseen tutkimukseen osallistui noin 800 satunnaisesti valittua tervettä varusmiestä, joiden seerumin 25OHD –

pitoisuus, paino, pituus, BMI ja kuntoindeksi määritettiin. Tämän jälkeen tutkimukseen osallistuvia seurattiin 90 päivän ajan ja rasitusmurtumat kirjattiin. 756 varusmiestä oli mukana koko tutkimuksen ajan ja näistä 22:lla voitiin todeta radiologisesti varmistettu rasitusmurtuma (2.9 %). Rasitusmurtuman insidenssi oli 11.6 (95 % CI: 6.8-16.5) 100 henkilövuotta kohti. Lopullisen monimuuttuja-analyysin mukaan niiden varusmiesten, joilla seerumin 25OHD -pitoisuus oli <76 nmol/l (mediaani), riski saada rasitusmurtuma oli 3.6 –kertainen (95 % CI: 1.2-11.1). Monimuuttuja-analyysissä BMI, ikä, lihaskuntoindeksi, 12 minuutin juoksutesti ja tupakointi eivät korreloineet rasitusmurtumien esiintyvyyteen. D-vitamiinipitoisuuden alhaisuus näyttää olevan rasitusmurtuman keskeinen riskitekijä.

D-vitamiinilla on osoitettu olevan myös vaikutuksia elimistön immuunipuolustukseen. Tutkimusten mukaan D-vitamiinilla on keskeinen rooli etenkin elimistön synnynnäisen immuniteetin toiminnassa. Siten D-vitamiinipitoisuudella saattaa olla merkitystä mm. hengitystieinfektioiden esiintyvyyteen. Kolmannessa osatyössä selvitimme D-vitamiinin vajauksen ja hengitystieinfektioiden insidenssin l. ilmaantuvuuden yhteyttä varusmiehillä. Tutkimukseen osallistui n. 800 suomalaista varusmiestä, jotka palvelivat Porin prikaatissa. Seerumin 25OHD –pitoisuudet määritettiin varusmiespalveluksen alussa heinäkuussa 2002 ja tutkimukseen osallistuvien hengitystieinfektiot kirjattiin 6 kuukauden ajan. Seerumin 25OHD –pitoisuuden keskiarvo oli 80 nmol/l heinäkuussa 2002 (n=756). Ne varusmiehet, joiden seerumin 25OHD –pitoisuus oli <40 nmol/l, olivat enemmän poissa palveluksesta hengitystiesairauden vuoksi. Tulos on tilastollisesti merkittävä ja siinä on huomioitu tupakoinnin vaikutus. Lisäksi tutkimuksessa osoitettiin, että säännöllisesti liikkuvilla oli korkeammat ja tupakoivilla alemmat D-vitamiinipitoisuudet. Tutkimuksen mukaan alentunut D-vitamiinipitoisuus lisää merkittävästi alttiutta sairastua hengitystieinfektioihin.

Neljännessä osatyössä tutkimme D-vitamiinisuplementaation vaikutusta varusmiesten hengitystienfektioiden ilmaantuvuuteen. Tässä satunnaistetussa kontrolloidussa tutkimuksessa (RCT) oli mukana 164 varusmiestä, jotka saivat 10 mikrog D-vitamiinia tai vastaavasti plaseboa päivittäin lokakuusta maaliskuuhun. Tutkimuksessa huomioitiin tupakoinnin vaikutus. D-vitamiiniryhmän keskimääräinen seerumin 25OHD –pitoisuus oli 79 nmol/l lokakuussa 2005 ja 72 nmol/l maaliskuussa 2006. Vastaavasti plaseboryhmän pitoisuudet olivat 74 nmol/l ja 51 nmol/l. D-vitamiini- ja plaseboryhmän välillä ei ollut tilastollisesti merkittävää eroa, kun tarkasteltiin poissaoloa palveluksesta hengitystieinfektion vuoksi (päämuuttuja). Sen sijaan D-vitamiiniryhmässä (51 %) oli tilastollisesti merkittävästi enemmän niitä, joilla ei ollut lainkaan poissaoloja hengitystienfektion vuoksi kuin plaseboryhmässä (36 %, p=0.045). Lisäksi Coxin monimuuttuja-analyysin mukaan D-vitamiiniryhmässä todettiin alhaisempi riski sairastua hengitystieinfektioon kuin plaseboryhmässä (HR, 0.71; 95 % CI 0.43-1.15). Tutkimus osoittaa, että D-vitamiinin suplementaatio saattaa vähentää hengitystieinfektioiden ilmaantuvuutta. Laajemmat kliiniset tutkimukset suuremmilla annoksilla ja tutkimuspopulaatioilla ovat kuitenkin tarpeellisia tuloksen varmentamiseksi.

ABBREVIATIONS

AMP	antimicrobial peptide
ANOVA	analysis of variance
ARTI	acute respiratory tract infection
BMC	bone mineral content
BMD	bone mineral density
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CYP27A1	cytochrome P450 vitamin D3 25-hydroxylase
CYP27B1, 1α-hydroxylase	cytochrome P450 25-hydroxyvitamin D3, 1alpha-hydroxylase
CYP24, 24-hydroxylase	cytochrome P450 25-hydroxyvitamin D3, 24-hydroxylase
D2	ergocalciferol
D3	cholecalciferol, calciol
DBP	vitamin D binding protein
7-DHC	7-dehydrocholesterol, provitamin D
e.g.	exempli gratia (meaning ‘for example’)
i.e.	id est (meaning ’that is’)
mRNA	messenger RNA
PLC	phospholipase C
PTH	parathyroid hormone
RCT	randomised controlled trial
RIA	radioimmunoassay
RXR	retinoid X receptor
SD	standard deviation
TCR	T cell antigen receptor
TLR	toll-like receptor
UV	ultraviolet
UVA	ultraviolet A
UVB	ultraviolet B
UVC	ultraviolet C
VDR	vitamin D receptor
VDR KO	vitamin D receptor knockout
VDRE	vitamin-D-responsive element
vs.	versus
25OHD	25-hydroxyvitamin D
25OHD2	25-hydroxyvitamin D2
25OHD3	25-hydroxyvitamin D3, calcidiol

1,25(OH)2D3	1,25-dihydroxyvitamin D3, calcitriol
1,24,25(OH)3D3	1,24,25-trihydroxyvitamin D3
24,25(OH)2D3	24,25-dihydroxyvitamin D3

INTRODUCTION

Vitamin D is produced in the skin from 7-dehydrocholesterol through a photolytic reaction induced by ultraviolet B (UVB) radiation from sun exposure. It is a precursor of the hormone 1,25(OH)2D that is formed in two hydroxylation reactions, first in the liver and then in the kidneys or in target organs (Holick et al. 1980). Vitamin D regulates calcium and phosphorous homeostasis in the body the process that is essential for bone mineralisation (Holick 2003). Clinically, vitamin D deficiency is known to lead to secondary hyperparathyroidism, causing rickets in children and both osteomalacia and osteoporosis in adults (Compston 1998).

Vitamin D status is determined through measurement of the major circulating form of the hormone, serum 25OHD (Utiger 1998). The scientific consensus is that vitamin D sufficiency can be defined as serum 25OHD >75 nmol/l (Dawson-Hughes et al. 2005). Vitamin D insufficiency is a global issue that can be regarded as epidemic among adults without sufficient sunlight exposure. At high northern latitudes, diet is the most important source of vitamin D during winter, because of inadequate sunlight exposure. Vitamin D insufficiency is commonplace in all age groups in the wintertime in Finland (Laaksi 2011). Since February 2003, vitamin D fortification of liquid milk products and margarines has been implemented in Finland. Furthermore, the Finnish National Nutrition Board renewed the recommendation of vitamin D fortification in April 2010.

Vitamin D regulates gene expression by vitamin D receptors (VDRs). An active form of vitamin D binds to the VDR that dimerises with the retinoic X receptor. The complex then binds to vitamin-D-responsive elements inside vitamin D–responsive genes (Rachez et al. 2000). Vitamin D contributes to bone health by increasing both intestinal calcium absorption and renal calcium reabsorption (Utiger 1998). Vitamin D is also known to decrease parathyroid hormone (PTH) secretion and enhance the differentiation of both osteoblast and osteoclast precursors. Thus it has a major effect on bone mineralisation and peak bone mass (Välimäki et al. 2004). In a randomised controlled trial (RCT), a rise in the mean 25OHD level from 53 to 74 nmol/l through vitamin D supplementation was shown to reduce fracture risk at the hip, forearm, and spine in people >65 years of age (Trivedi et al. 2003). In another study, vitamin D inadequacy was linked to increased risk of hip and other nonvertebral fractures (LeBoff et al. 1999). The findings support the hypothesis that vitamin D inadequacy predisposes young Finnish men to bone stress fractures, a common overuse injury in athletes and in those performing their military service.

Toll-like receptors (TLRs), monitoring the host for the presence of pathogens, are stimulated by pathogens. This stimulation upregulates VDR

expression and vitamin D hydroxylase genes, leading to the production of antimicrobial peptides (Wang et al. 2004). Further, the induction of cathelicidin messenger RNA (mRNA) was shown to be lower in the presence of serum from African Americans, which contains less 25OHD than does serum from Caucasians (Liu et al. 2006). In the case of respiratory pathogens accumulated in the airways, increased activation of vitamin D and then increased levels of cathelicidin mRNA have been shown in another study. Again, respiratory epithelial cells can activate vitamin D and create a microenvironment with high levels of the active form of the vitamin D upregulating the cathelicidin antimicrobial peptide gene, an important component of innate immunity in the lungs. Local vitamin D activation may be an important component of host defence. (Hansdottir et al. 2008; Hansdottir et al. 2011) This hypothesised mechanism could also explain the differences in proneness to acute respiratory tract infections in young Finnish men during their military service.

REVIEW OF THE LITERATURE

Vitamin D

History

The importance of sunlight for human health was recognised by Hippocrates in ancient Greece: he believed that the southern side of the hill, receiving the most sunlight each day in the northern hemisphere, was the healthiest place to live.

In the early 17th century, Francis Glisson, Daniel Whistler, and Arnold de Boot discovered rickets, a bone disease in children that is characterised by a poorly mineralised and deformed skeleton. Later, in the early 19th century, Jędrzej Śniadecki noticed that children living in the city of Warsaw had high incidence of rickets whilst children in rural regions did not develop the disease. He thought that increased exposure to sunlight in the countryside prevented these children from developing rickets. Further, in the late 19th century Theodore Palm noted that children living in equatorial countries did not develop rickets. It was then found that rickets incidence has a geographical background related to sunlight exposure. (Holick 1994)

In the late 19th century, Niels Finsen exposed subjects with cutaneous tuberculosis to artificial sunlight when working to find a cure for tuberculosis. He found that producing moderate sunburn to a small area of affected skin with intense light caused the superficial skin layer to peel away but exposed normal and healthy skin underneath. This phototherapy improved the skin in almost all cases. Later it was used routinely as a treatment for pulmonary tuberculosis, one for which Finsen was awarded a Nobel Prize in 1903. (Zasloff 2006)

In 1919, Edward Mellanby reported that a restrictive diet led to rickets in dogs. Investigating the cause of rickets and seeking a cure, he found an exclusively oat-based diet to induce rickets. In addition, he found that adding cod-liver oil to the diet cured the dogs within a few months, so he concluded that a component of cod-liver oil (that oats do not contain) was essential in preventing rickets. The dogs in Mellanby's experiments were raised indoors with no exposure to sunlight. This led to a historical accident: vitamin D became classified as a vitamin and not as a hormone – i.e., a compound that is synthesised in the body and acts as a chemical messenger from one cell, or group

of cells, to another – in contrast to a vitamin as a biologically active organic compound that is not synthesised in the body but obtained solely through the diet. Cod-liver oil entered systematic use in Germany in 1823 and soon after that also in Finland as a folk medicine. (Holick 1994)

In 1922, Elmer McCollum discovered the compound vitamin D. He investigated the chemical composition of cod-liver oil, which was known to prevent night blindness and fractures. He was anxious to find out whether cod-liver oil retained its properties when heated and oxygenated. This was tested through heating and oxygenation of cod-liver oil. It was found to prevent fractures still but no longer night blindness, meaning that two different active compounds are involved. The compound destroyed this way was named vitamin A, and the heat-stable component became known as vitamin D. (Mohr 2009)

In 1923, Harry Goldblatt and Katherine Soames established that when 7-dehydrocholesterol is irradiated with UV light, a form of a fat-soluble vitamin is produced. Alfred Hess and Mildred Weinstock further substantiated this to be vitamin D. They also showed that wheat, lettuce and vegetable oils displayed antirachitic activity after exposure to UV irradiation. In 1924, Harry Steenbock found that animals were able to produce vitamin D and suggested that it was not a dietary constituent but UV light that was essential in protection against rickets. Steenbock concluded that irradiation of food substances is beneficial for protection against rickets in children. (Steenbock 1924; Holick 1994)

Adolf Windaus received a Nobel Prize in 1928 for the discovery that exposure to UV was responsible for vitamin D synthesis. He showed that ultraviolet light has an isomerising effect on ergosterol, a steroid component of fungal-cell membranes. He was able to determine the chemical structure of vitamin D; vitamin D2, produced by ultraviolet irradiation of ergosterol, was characterised in 1932, and the discovery of the precursor of vitamin D3 (7-dehydrocholesterol, 7-DHC) occurred in 1937, when it was noticed that only those foods containing cholesterol could cure rickets after being irradiated with UV light. Windaus also showed that the antirachitic component of cod-liver oil was identical to vitamin D3. Soon after the structure of vitamin D was characterised and a process developed for its synthesis, vitamin D was directly added to milk, making ultraviolet-irradiation of milk unnecessary. (Mohr 2009)

In the early 20th century, rickets was a significant world health problem. In industrialised cities of northern Europe and the north-east United States, it was epidemic and >80% of young children who died (of whatever cause) had clinical manifestations of rickets (Holick 1994). As it had been shown that rickets could be cured by exposure to sunlight or ingestion of UV-irradiated food, there were two competing concepts in the prevention of rickets (McCollum et al. 1995).

Metabolism and mechanism of action

The chemical structure of vitamin D is closely related to that of classic steroid hormones (Norman 2008). There are two forms of vitamin D: the main source in humans is skin photosynthesis following exposure to sunlight producing vitamin

D3 (cholecalciferol), and the other is vitamin D2 (ergocalciferol) produced by ultraviolet radiation in a variety of plant materials and yeasts (Norman 1998). Sunlight is the main source of vitamin D and usually contributes up to 90% of serum concentration of vitamin D. The remaining 10% comes from dietary intake and vitamin D supplements. In fur-bearing animals and many birds, sunlight is not able to reach the skin; vitamin D is synthesised in their fur or feathers, and these animals then eat the resulting cholecalciferol by licking the fur or rubbing the beak on the feathers. (Lehmann et al. 2010)

Solar UV radiation consists of UVA, UVB, and UVC, depending on the wavelength of the photons. Almost all UVC photons are usually absorbed completely by the ozone layer and do not reach the earth at all. In the two innermost layer of the skin's epidermis, the stratum basale and stratum spinosum, solar ultraviolet-B radiation at a wavelength of 290–315 nm, with its maximum spectral effect at 297 nm, penetrates the skin and converts 7-DHC (provitamin D3) to previtamin D3. Less than 1% of previtamin D3 is formed at UVA wavelengths. The action spectra for erythema and vitamin D3 production are similar, but maximal synthesis is achieved at suberythemogenic UVB doses, meaning a few minutes of summer sun exposure (MacLaughlin et al. 1982; Wolpowitz et al. 2006). Longer exposure does not increase one's stores of vitamin D but does increase the possibility of DNA damage. Approximately two thirds of human cutaneous 7-dehydrocholesterol can be found in the epidermal layer of the skin, the remaining third being in the dermis (Lehmann et al. 2010). The amount of previtamin D3 is less than 15% of the substrate 7-DHC available (Webb et al. 1988). In ageing, the amount of 7-DHC begins to decline; when exposed to the same amount of sunlight, a person who is 70 years of age makes only ¼ of the vitamin D that a 20-year-old person can make (MacLaughlin et al. 1985; Holick et al. 1989).

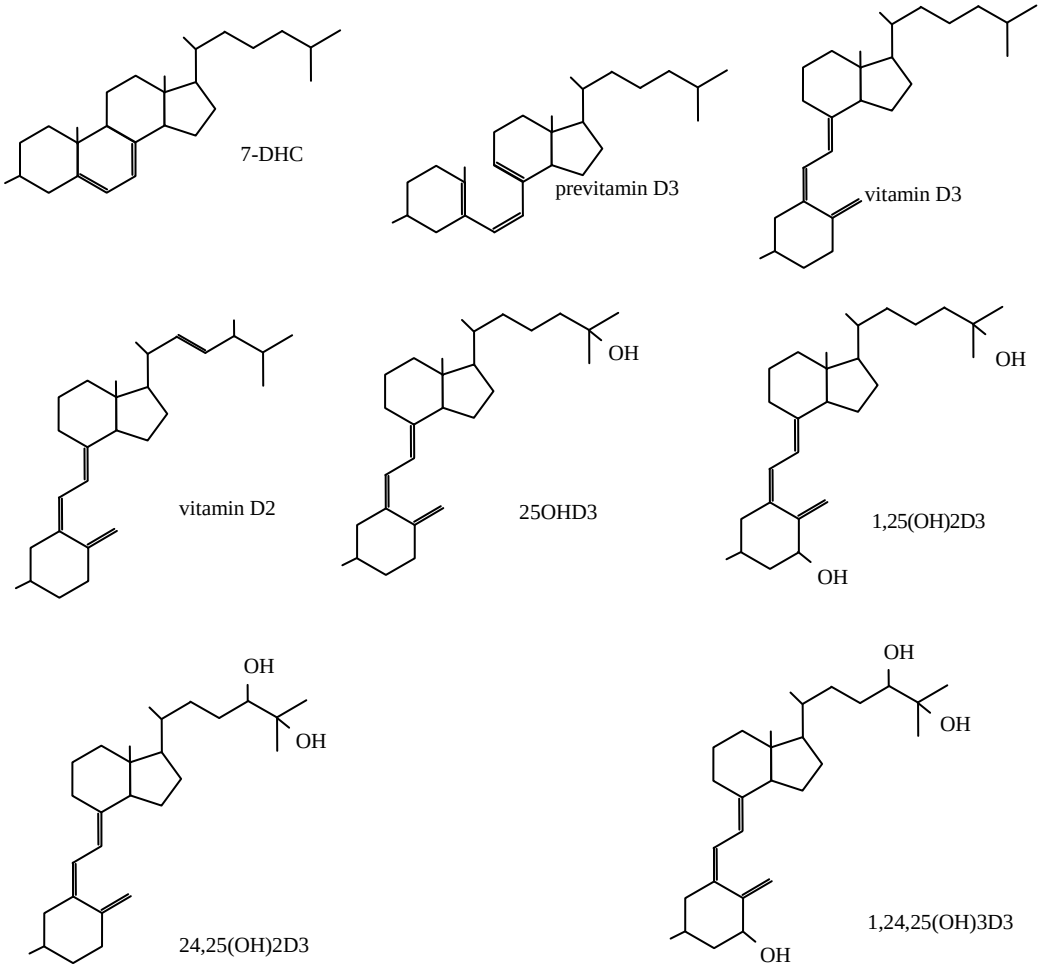
Previtamin D3 is thermodynamically unstable and is isomerised to form vitamin D3 by a non-enzymatic and highly temperature-dependent process. The formation of vitamin D3 is relatively rapid, reaching a maximum within 12-24 hours after UVB exposure. Half of the previtamin D3 can isomerise to vitamin D3 within 2.5 hours in the skin. Vitamin D3 moves to the extracellular space and binds to vitamin D binding protein (DBP), entering the circulation. (Lehmann et al. 2010)

Both the intensity of the UV radiation and the level of skin pigmentation regulate the rate of previtamin D3 formation but not the maximal level achieved. The intensity of ultraviolet radiation from sunlight varies with the season, latitude, and time of day. The farther from the equator, the less solar exposure to produce D3 there is in the course of a year, as the rays enter at a more oblique angle (zenith angle) and more UVB photons are absorbed by the ozone layer, because of the greater distance. Generally, at a latitude below 37°, vitamin D synthesis occurs in the skin throughout the year. On the other hand, in the early morning or late afternoon, vitamin D3 production is ineffective in the skin even in summer, because of the oblique zenith angle. (Webb et al. 1988; Holick 2004) Both clothing and use of sunscreen prevent vitamin D3 production (Matsuoka et al. 1987; Matsuoka et al. 1992). Melanin is an effective natural sunscreen that

absorbs UVB photons. People with greater skin pigmentation require longer sunlight exposure for vitamin D3 production. (Jörgensen et al. 2010)

In the event of excess UVB radiation, previtamin D3 will be degraded into inactive sterols lumisterol and tachysterol, or be retransformed to 7-DHC. In addition, vitamin D3 will be converted to inactive suprasterols I and II and 5,6 transvitamin D3, with avoidance of vitamin D intoxication. (Holick et al. 1979; Holick et al. 1980; Holick et al. 1981)

Nutritional vitamin D from the diet or dietary supplements is transported from the intestine in chylomicrons via lymph veins and released into the liver (Bouillon et al. 1998). Both vitamin D synthesised in the epidermis and from the diet or dietary supplements are biologically inert and require hydroxylations by mitochodrial P450s in order to become active. Firstly, vitamin D is hydroxylated by 25-hydroxylase (CYP27A1) present in the liver to form 25-hydroxyvitamin D3 (25OHD3), the major circulating form of vitamin D. The production of 25OHD3 is not significantly regulated but is mainly dependent on substrate concentration, and approximately 40% of vitamin D3 is converted into 25OHD3 (Kato 2000). After hydroxylation, 25OHD3 enters the circulation. It has a half-life of about 15 days (Jones 2008).



The 25OHD3 is transported to the kidneys for successive hydroxylation by 1α-hydroxylase (CYP27B1) to form the most biologically active form of vitamin D, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and finally to target tissues for biological response (Holick et al. 1980; Norman 1998). The serum level of 1,25(OH)2D3 is usually 75–200 pmol/l, being a thousandth that of 25OHD3 (25–200 nmol/l). The serum half-life of calcitriol is 10–24 hrs (Levine et al. 1985). There is evidence that also 25OHD3 has hormonal activity (Lou et al. 2004).

The expression of CYP27B1 has been documented in extrarenal tissues also: the skin, macrophages, lymph nodes (Hewison et al. 2000), normal and malignant colon tissue (Matusiak et al. 2005), and normal and malignant breast tissue (McCarthy et al. 2009), among other tissue types. This hydroxylation is tightly upregulated by parathormone (i.e., PTH), hypocalcaemia, and hypophosphataemia and downregulated by hypercalcaemia, hyperphosphataemia, and 1,25(OH)2D3 itself (Hewison et al. 2000).

Inactivation of 25OHD3 and/or 1,25(OH)2D3 is catalysed by a mitochondrial P450 (CYP24A) that 24-hydroxylates these to form 24,25(OH)2D3 or by 1α,24,25(OH)3D3 principally in the proximal tubules of the kidneys (Guryev et al. 2003).

There has been an assumption of equivalence of vitamin D2 and D3, as these forms differ only in the side chain to the sterol skeleton. However, in recent studies orally administered vitamin D3 has increased serum 25OHD up to 1.7 times more efficiently than does vitamin D2 when administered in molarly equal amounts for two weeks. In a study with single doses of vitamin D3 or vitamin D2, they led to equal increase in serum 25OHD levels, but those levels persisted or even continued to rise after three days in the vitamin D3 group while they declined and reached the baseline value in two weeks in the vitamin D2 group (Trang et al. 1998; Armas et al. 2004). Vitamin D2 has been shown to have a diminished ability to bind to DBP in the blood and a shorter shelf life as a supplement (Houghton et al. 2006). There is a trend to replace vitamin D2 with vitamin D3 in fortified dairy products or supplementation (Thacher et al. 2010).

Biological actions of vitamin D are mediated through a vitamin D receptor that belongs to the nuclear hormone receptor family. This has been found in cells involved in maintaining calcium homeostasis but also in others, such as a number of cancer cells (Christakos et al. 2011). As bound to 1,25(OH)2D3, the VDR forms a heterodimer with a retinoid X receptor (RXR). This complex acts as a transcription factor depending on the coregulatory proteins that are attached to the complex. These complexes will be bound to vitamin D response elements in the promoter region of target genes in which they modulate their transcription. (Kato 2000; Goltzman 2010)

In addition, as other hormones do, vitamin D mediates its effects partially through nongenomic actions by activating several signal transduction systems. In genomic action, the regulation of gene expression by vitamin D is mediated by VDRs and takes place within hours. It is likely that nongenomic response is mediated by a specific membrane-bound VDR and occurs within seconds to minutes. Increases in intracellular calcium levels, stimulation of intestinal

calcium transport flux, and activation of protein kinase C are examples of the nongenomic action of vitamin D. (Lehmann et al. 2010)

Physiological actions of vitamin D

Effect on bone and calcium metabolism

The principal function of vitamin D is to maintain mineral homeostasis both by increasing calcium and phosphorus absorption from the intestine and by enhancing their renal reabsorption (Holick 2003). If normal serum calcium cannot be maintained by intestinal absorption, increased PTH secretion mobilises bone calcium and induces the synthesis of 1,25(OH)2D3. In children, vitamin D deficiency inhibits the calcification of cartilage and causes rickets. In adults, by contrast, the bone matrix (the osteoid) is not mineralised, causing osteomalacia and osteoporosis due to a reduction in bone mass. An increased risk of hip and other fractures in adults is a known consequence of vitamin D deficiency. (Compston 1998; Utiger 1998; Lips et al. 2011)

A major consequence of the loss of VDR activity is a defect in intestinal calcium and phosphate absorption, which is the primary cause of decreased bone mineralisation. For example, the phenotype of VDR-knockout (KO) mice includes rickets, osteomalacia, and increased PTH secretion, but when the mice are fed a high-calcium, high-phosphorus diet, calcium and PTH levels are normalised and osteomalacia and rickets are prevented. It is clear that vitamin D is vital for mineral homeostasis. (Christakos et al. 2011)

Transcellular transport of calcium is activated by 1,25(OH)2D3 in the duodenum, where VDR is expressed in the highest concentration and upregulates genes responsive for transportation of calcium. Most of the absorption at low and moderate levels of calcium is due to this active transcellular transport. During higher calcium intake, calcium is also absorbed in the intestine by passive diffusion paracellularly (Heaney et al. 2005). Usually calcium intake and absorption are directly related, but when calcium intake is lowered, the percentage from calcium intake that is absorbed can increase from 25% to 37% because of increased serum concentrations of PTH and 1,25(OH)2D3 (Dawson-Hughes et al. 1993; Hunt et al. 2007). A change in serum 25OHD concentration from 50 to 86 nmol/l has been shown to cause a statistically significant increase in calcium absorption in the intestine (Heaney et al. 2003).

Vitamin D has also been proved to have direct effects on bone. *in vitro* studies have shown that 1,25(OH)2D3 can stimulate the formation of osteoclasts by upregulating osteoclast differentiating factor (Margolis et al. 2010).

Other actions of vitamin D

Vitamin D may also have rather interesting and important non-classical actions. The active form of vitamin D, 1,25(OH)2D, has been shown to promote insulin secretion, inhibit cell proliferation, and stimulate cell differentiation (Bikle 2008). Vitamin D has apoptotic effects on prostate cancer cells *in vitro*. It has effects on cancer invasion and angiogenesis (Ylikomi et al. 2002). In epidemiological studies, vitamin D has been negatively associated with breast and colon cancers (Cannell et al. 2006). Also, vitamin D deficiency in infancy has been linked to diabetes (Hyppönen et al. 2001; Oilinki et al. 2012), hypertension, multiple sclerosis (Correale et al. 2009; Pierrot-Deseilligny et al. 2010), and some other cancers (Holick 2004). Vitamin D insufficiency has also been associated with depression in men and with severe asthma exacerbation in childhood (Brehm et al. 2010; Lee et al. 2011).

Vitamin D metabolites have clinical utility in that 1,25(OH)2D3 and analogues are successfully used for treatment in cases of, for example, hyperproliferative skin disease and psoriasis (Guryev et al. 2003).

The role of vitamin D in immunity

Innate immunity is one part of the first-line barriers in host defence and responds rapidly to microbes that operate as an activator of the system. Antimicrobial peptides (AMPs) are evolutionarily ancient weapons in the battle against bacteria, viruses, and fungi that are available for activation even before the body has recognised an antigen (Zasloff 2002). AMPs have been found both in epithelial tissues and in phagocytic blood cells, in which they damage the lipoprotein membranes of microbes (Laube et al. 2006). In the moist airways, these proteins are secreted as a thin layer of fluid lying above the apical surface of the epithelium and below the mucous layer covering the epithelial surface. Binding of microbes to the epithelium provokes the expression of AMPs, creating a barrier that is chemically lethal to microbes. Some AMPs are secreted constitutively but others, such as LL-37 (cathelicidin), as a response to stimuli from microbial components (Zasloff 2002). Cathelicidin acts also as an attractant for macrophages and neutrophils (Laube et al. 2006).

Interaction with the immune system is one of the non-classic effects of vitamin D. Liu et al. were able to show that synthesis of vitamin D by sunlight exposure upregulates the expression of AMPs. Specifically, the stimulation of toll-like receptors, which monitor the host for the presence of microbe antigens, by pathogens upregulates the expression of two genes in monocytes – a VDR and CYP27B1, converting 25OHD3 to active 1,25(OH)2D3 – and leads to the production of AMPs. The incubation of activated monocytes with 1,25(OH)2D3 produce cathelicidin in a dose-dependent manner. (Liu et al. 2006) Cathelicidin concentrated in phagocytic vacuoles destroys M. tuberculosis in *in vitro* conditions, and this effect is more pronounced after macrophages are exposed to

1,25(OH)2D3. The gene-encoding cathelicidin has a vitamin-D-responsive element (VDRE) within the promoter region, and it is positively regulated by vitamin D (Wang et al. 2004). This gene and CYP27B1 are both expressed by the airway epithelium, indicating that active vitamin D can be produced locally within the lungs, leading to the production of cathelicidin (Hansdottir et al. 2011). Local vitamin D activation might be an important component of innate immunity in the lungs (Hansdottir et al. 2008).

Vitamin D regulates the activation of human T cells, controls T cell antigen receptor signalling, and enhances the recognition of microbe antigens by T lymphocytes. Human T cells have very low expression of signalling proteins (phospholipase C), correlating with low T cell antigen receptor (TCR) responsiveness. TCR triggering upregulates PLC expression and correlates with greater TCR responsiveness and T cell activation. The induction of PLC is dependent on vitamin D and expression of VDRs as a VDR bound to 1,25(OH)2D3 activates the gene-encoding PLC. (von Essen et al. 2010)

Vitamin D and respiratory tract infections

The respiratory mucosal epithelia, including those of the middle ear and pharyngotympanic tube, secrete antimicrobial peptides as part of innate immunity that inhibit the growth of otitis media pathogens: Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae (Lee et al. 2004; Vlaminckx et al. 2005).

Higher vitamin D status might protect against influenza. The prevalence of respiratory infections showed a strong seasonal pattern inversely related to 25OHD concentrations in a cohort study with 7,000 over-45-year-old participants. Furthermore, after adjustment for adiposity, lifestyle, and socio-economic factors, each 10 nmol/l rise in serum 25OHD concentration was associated with a 7% lower risk of infection in the study. (Berry et al. 2011)

In a secondary analysis of the Third National Health and Nutrition Examination Survey with almost 19,000 participants, serum 25OHD concentrations were found to be inversely associated with recent upper respiratory tract infection (URTI) after adjustment for season, body mass index (BMI), smoking, asthma, and chronic obstructive pulmonary disease (COPD). An independent association between lower serum 25OHD and URTI was revealed and URTI was reported by 24% of subjects with 25OHD <25 nmol/l, by 20% with 25OHD 25–75 nmol/l, and by 17% with serum 25OHD >75 nmol/l. (Ginde et al. 2009)

There are some randomised placebo-controlled and double-blind trials of vitamin D supplementation for prevention of respiratory tract infections in adults. A lower rate of self-reported URTI or influenza was observed in the intervention arm as compared to the placebo group in a trial with 208 healthy postmenopausal African American women who were given 20 µg of vitamin D3 daily or a placebo for two years. After the increase of vitamin D3 dosage from 20 µg to 50 µg daily or a placebo for 12 months, the effect was magnified. (Aloia

et al. 2007) A follow-up study with 162 healthy adults given 50 µg of vitamin D3 or a placebo daily for 12 weeks showed no benefit with respect to the incidence and the severity of URTI symptoms for the vitamin D group as opposed to the placebo group. However, there was a statistical tendency to favour the vitamin D group.(Li-Ng et al. 2009) Further, Avenell et al. failed to show a statistically significant difference between the vitamin D and placebo group in the secondary end point of self-reported infection rate in a study with 3,500 elderly subjects who were given 20 µg of vitamin D3 or a placebo for more than two years. The study was part of the Randomised Evaluation of Calcium or Vitamin D (RECORD) trial. (Avenell et al. 2007)

Stress fractures

Fractures induced by cyclic loading of normal bones with abnormal forces are described as stress fractures (Anderson et al. 1996). These fractures are common overuse injuries in military populations. At the beginning of Finnish military service, all conscripts perform eight-week basic training, with gradually increasing levels of strain. When completing this training, they are expected to maintain their fitness for battle after a 15 km march or ski journey, with a 25 kg military pack, on two consecutive days. In a study of Finnish male military recruits, 15 of the 179 recruits (8.4%) developed a stress fracture during military service (Välimäki et al. 2005). In the US Army's basic combat training (8 weeks), the reported incidence of stress fractures among male trainees has ranged from 0.9 to 5.2% (Jones et al. 2002). Still, the prevalence of these fractures can be up to 49% in military populations; it is largely dependent on the physical demands of training (Niva et al. 2009).

Stress fractures occur especially near the beginning of military service, when the bones are not adapted to increased mechanical loads. Anatomically, the stress fractures are found mainly (95%) in the lower extremities: the tibial shaft and metatarsal bones. Stress fractures of the pelvis, hip, thigh, and knee are rare but are among the high-risk injuries associated with a tendency for protracted healing and complications such as dislocation of the femoral neck leading to avascural necrosis. (Pihlajamäki et al. 2006; Mattila et al. 2007)

Stress fractures occur as a result of microdamage after repetitive strains to a normal bone (Daffner et al. 1992). Bone is dynamic tissue and is able to adapt its shape and size in response to excessive strain. Bone remodelling is cyclic removal of bone by osteoclasts (resorption) followed by the deposition of new bone by osteoblasts (formation). In bone modelling, the bones are reshaped again by the activation of osteoblasts and osteoclasts. These bone-strengthening processes may last up to six months, and up to 10% of the skeleton undergoes remodelling each year. Unloading of the skeleton will follow rapid bone loss and osteoporosis. (Kiuru et al. 2004; Brandi 2009)

Both a poor aerobic fitness level (Shaffer et al. 1999) and low muscle strength (Beck et al. 2000) at the start of a physical training programme,

increased age (Gardner et al. 1988), female sex (Jones et al. 2002), smoking (Altarac et al. 2000), high or low foot-arch height (Simkin et al. 1989), valgus knees (‘knock-knees’) (Cowan et al. 1996), and low bone mineral density (Pouilles et al. 1989) are some risk factors for development of stress fractures among military populations.

Localised pain after a recent increase in physical training activity is a common symptom of a stress fracture. Usually, the pain eases during rest. Although the clinical findings may be exiguous, tenderness upon palpation and localised oedema might be found. One-leg standing or jumping may reveal high-risk femoral-neck stress fracture. (Kiuru et al. 2004)

For the diagnosis of a stress fracture, plain radiography should be performed initially and repeated after two weeks. In the case of urgent diagnosis for high-risk stress fractures, magnetic resonance imaging needs to be used for its higher sensitivity. (Kiuru et al. 2004; McClung et al. 2010) Treatment of stress fractures includes rest and cessation of the physical activity for 3-8 weeks, depending on the severity and the localisation of the fracture. Return to physical training should be gradual. High-risk stress fractures require surgical consultation and surgery. The prognosis with non-invasive and conservative treatment is usually excellent for non-displaced-femoral-neck stress fractures, though the use of non-weight-bearing crutches is essential. In contrast, the displacement of a femoral-neck stress fracture is treated surgically, because of the risk of serious complications such as avascular necrosis. (Kiuru et al. 2004)

Vitamin D as a factor for prevention of stress fractures

Calcium and phosphate homeostasis has a key role in bone mineralisation and peak bone mass development. Vitamin D is known to contribute to this homeostasis by increasing intestinal calcium absorption, enhancing renal calcium reabsorption, decreasing parathyroid hormone secretion, and affecting both osteoblast and osteoclast differentiation (Holick 2004).

With serum 25OHD concentration below 78–90 nmol/l, there is an increase in serum PTH, showing vitamin D insufficiency (Krall et al. 1989; Chapuy et al. 1997). The increased PTH increases bone turnover and fragility (Szulc et al. 2003). Importantly, in a recent northern European study with about 700 subjects pathologic mineralization defects of bone were seen in crista iliaca biopsies of those with serum 25OHD <75 nmol/l (Priemel et al. 2010). Low 25OHD levels are associated with reduced bone mineral content (BMC) and cortical thickness of the femoral neck in young men (Szulc et al. 2003; Välimäki et al. 2004). In addition, vitamin D supplementation increases bone mineral density (Baeksgaard et al. 1998). In the population study with >13,000 individuals including both younger and older adults BMD increased continuously with higher 25OHD levels. Interestingly, this rise was seen throughout the reference-range of 22.5-94 nmol/l contributing to the development of higher peak bone mass in young men. (Bischoff-Ferrari et al. 2004) Clinically, low vitamin D status is a determinant of increased osteoporotic fracture risk (Lips et al. 2010).

The number of prospective studies of vitamin D insufficiency and stress fractures is quite limited. In a study of over 2,500 Israeli soldiers, 25OHD levels were lower in patients with scintigraphically proved high-grade stress fractures (63 nmol/l) than in control subjects (75 nmol/l) (Givon et al. 2000). In the case of serum 25OHD <50 nmol/l, the risk of stress fracture of the tibia and fibula was double that of those with serum 25OHD >100 nmol/l in a cohort study of 1,200 female US Navy recruits. That study also found an inverse dose–response gradient between 25OHD and stress-fracture risk. (Burgi et al. 2011)

The results as to the association between low calcium intake and stress fracture development are controversial (Myburgh et al. 1990; Cline et al. 1998). In a Finnish study with 220 male conscripts, PTH levels were higher in those who developed stress fractures than among controls. There was no statistically significant difference in the vitamin D status of the groups, though (Välimäki et al. 2005).

In a randomised double-blind and placebo-controlled intervention trial of vitamin D supplementation for the prevention of stress fractures with >5,200 female US Navy recruits, the participants were given either 2,000 mg of calcium and 20 μg of vitamin D or a placebo daily during eight weeks' basic training. The intervention reduced the incidence of stress fractures by 20%, according to intention-to-treat analysis (Bouillon 2008; Lappe et al. 2008). Further, a recent study found that two polymorphisms in the VDR may independently increase the risk of stress fractures (Chatzipapas et al. 2009; McClung et al. 2010).

Vitamin D insufficiency

Serum 25OHD is the best marker for vitamin D status, since both high serum concentrations in the summer and low concentrations in the winter have been observed at northern latitudes, reflecting the amount of exposure to the sun (Chapuy et al. 1997). Vitamin D sufficiency can be detected from the increase in serum parathyroid hormone secretion; it starts rising at 25OHD cut-off levels of 78–90 nmol/l (Krall et al. 1989; Chapuy et al. 1997; Harkness et al. 2005). Additionally, it seems that the serum 25OHD threshold for PTH increase is lower in African Americans than among Caucasians (Aloia et al. 2010). Because of the relationship of skin pigmentation to vitamin D synthesis, serum 25OHD levels are highest in Caucasians, lowest in African Americans, and intermediate in Hispanics (Zadshir et al. 2005). On the other hand, PTH has an inverse relationship to skin pigmentation; African Americans have the highest and Caucasians the lowest plasma levels of PTH (Aloia et al. 2006).

Sunlight is the main source of vitamin D and produces it very effectively in skin cells: full-body exposure to UVB radiation of 15–20 minutes is able to produce up to 250 μg of vitamin D (Stamp et al. 1977). Of note is that 1 μg of vitamin D orally administered each day increases circulating serum 25OHD levels from 0.6 to 1.2 nmol/l (Heaney 2007).

In northern countries, sun exposure is inadequate for vitamin D production because of the greater distance through the atmosphere in wintertime (Norman 1998). Therefore, dietary intake and vitamin D supplements are sources of vitamin D from October to March in Finland. The foods that naturally contain substantial amounts of vitamin D are fish-liver oils, fish, mushrooms, and egg yolks. Among seafood, the lamprey, pike-perch, whitefish, Baltic herring, and salmon are excellent sources of vitamin D3, though, farmed salmon contain only ¼ the vitamin D3 found in wild salmon. (Lu et al. 2007; Lehmann et al. 2010)

Vitamin D insufficiency can be regarded as epidemic among adults with inadequate exposure to sunlight (Holick 2003). It is common in children during wintertime in Europe. In elderly people, it lasts throughout the year, and serum 25OHD3 concentrations in institutionalised people are even lower (McKenna et al. 1998; Schleithoff et al. 2006). Also, vitamin D insufficiency has been very commonplace among young men, young girls, and healthy adults in winter in Finland (Lamberg-Allardt et al. 2001; Välimäki et al. 2004). High prevalence of vitamin D insufficiency and secondary hyperparathyroidism have been found in African immigrants to Finland (Islam et al. 2012).

The fortification of liquid milk products (0.5 µg / 100 ml) and spreads (10 µg / 100 g) since February 2003, following the recommendation of the Finnish Ministry of Social Affairs and Health, has improved vitamin D status and increased vitamin D intake in Finland. However, approx. 20% of Finnish people still had inadequate vitamin D status in 2004 in winter, especially young girls, young adults, and middle-aged women (Lehtonen-Veromaa et al. 2008). It seems that vitamin D fortification of milk products did not resolve hypovitaminosis D in Finland (Välimäki et al. 2007). In April 2010, the Finnish National Nutrition Board recommended that vitamin D fortification be increased to 1 µg / 100 ml in liquid milk products and 20 µg / 100 g of fat spreads (but not butter) (Pietinen et al. 2010).

There is growing consensus on boundaries between stages of vitamin D inadequacy: <25 nmol/l for deficiency, 25 to 49.9 nmol/l for insufficiency, 50 to 75 nmol/l for hypovitaminosis, and >75 nmol/l for sufficiency (Dawson-Hughes et al. 2005). With these thresholds, over 90% of the more pigmented and around 75% of the Caucasian population in the USA are vitamin D insufficient. The prevalence of vitamin D insufficiency has also doubled in 10 years in these populations. (Adams et al. 2010)

Finally, American Endocrine Society recommends the screening of serum 25OHD in patients at risk for vitamin D deficiency. The society do not recommend population screening for vitamin D, though. Based on the Endocrine Society's consensus vitamin D deficiency is defined as a serum 25OHD below 50 nmol/l and vitamin D insufficiency as a serum 25OHD of 50–75 nmol/l. (Holick et al. 2011) On the other hand, the 2011 Report on Dietary Reference Intakes for Ca and Vitamin D from the Institute of Medicine in the USA concludes that scientific evidence supports a key role of vitamin D in skeletal health. However, for extraskeletal outcomes, including immune disorders, the evidence is still inconsistent. According to the report existing evidence suggests that vitamin D sufficiency is achieved at serum 25OHD levels of >50 nmol/l.

There is also evidence that very high values are not associated with greater benefit but U-shaped associations can be observed showing risks at both low and high levels of vitamin D for some outcomes (Ross et al. 2011).

AIMS OF THE STUDY

The aims of the present study were:

1. To study vitamin D status and the effect of vitamin D fortification as a national health policy on serum vitamin D concentrations in young Finnish men (I).
2. To study the association between bone stress fractures and low serum vitamin D concentrations in young Finnish men (II).
3. To study the association between vitamin D insufficiency and acute respiratory tract infections in young Finnish men (III).
4. To evaluate the effect of vitamin D supplementation on the prevention of acute respiratory tract infections in young Finnish men (IV).

MATERIAL, SUBJECTS, AND METHODS

Subjects and methods (I)

For the study, 196 Finnish men (18–28 yrs) undergoing military training in the Finnish Defence Forces as conscripts were recruited. A representative sample of subjects (n = 96) were recruited at health examinations covering all conscripts (n = 690) in January 2003. Similarly, 100 subjects were recruited, representing all of the conscripts (n = 1,158), in January 2004, nearly a year after the start of nationwide vitamin D fortification (February 2003). The volunteers were asked to participate in the examination a unit at a time, and each man was invited until the target number had been reached. Use of vitamin D supplements and staying in sunny southern countries in the last three months were used as exclusion criteria. The men all had passed their initial medical examination as healthy.

Serum 25OHD concentrations were determined with an OCTEIA® enzyme immunoassay by IDS (Immunodiagnostic Systems) in January 2003 (n = 96) and in January 2004 (n = 100), and serum 25OHD concentrations below 40 nmol/l were regarded as indicating vitamin D insufficiency. All volunteers provided written informed consent. The Ethics Committee of the Pirkanmaa Hospital District approved the study.

Subjects and methods (II, III)

For the prospective studies, 800 young Finnish men (range: 18–28 yrs; mean: 19.8 yrs) undergoing military training in the Finnish Defence Forces as recruits of the same infantry unit were randomly selected and enrolled for a study in July 2002. Because of failed samples, incomplete follow-up data, and interrupted military services, the total study population was composed of 756 subjects. They were receiving no medication, and their medical examination found them to be healthy. Those taking vitamin D supplements or having travelled in southern or sunny countries in the preceding three months were excluded from the study.

All Finnish men fall due for mandatory six-, nine-, or 12-month national service at the age of 18. Military service is voluntary for women. Annually, on

average, 26,500 male and 500 female recruits undergo military training. The military conditions with respect to physical activity, nutrition, clothing, living quarters, and exposure to sunlight were homogenous for the study population. All volunteers provided written informed consent. The Ethics Committee of the Pirkanmaa Hospital District approved the study.

Background information

A structured questionnaire was used to elicit information on age, smoking, amount of physical exercise before military service (hrs/wk), physician-diagnosed lactose-intolerance, education, geographical location (northern or southern Finland), and area of residence (urban or rural). Height and weight were measured to determine body mass index (in kg/m2).

The information about aerobic fitness and muscle strength used in this study was obtained during the first weeks of military service. Aerobic fitness was measured by means of a 12-minute running test. Muscle strength was assessed with five metrics (distance of a horizontal jump and number of sit-ups, push-ups, pull-ups, and back-lifts), with an index of 0 to 3 employed for each. Information about daily smoking was assessed from questionnaires.

Because most stress fractures occur during the first eight-week basic training period common to all military recruits, the subjects were followed up to identify possible stress injuries to bone for 90 days (Jones et al. 1989). The subjects were informed about the study and instructed to seek medical attention in the event that symptoms suggestive of bone stress injuries occurred. Those who by clinical examination with careful history-taking were suspected to have sustained a bone stress injury underwent plain radiographic imaging with accepted radiological assessment (Kiuru et al. 2004; Lappe et al. 2008). The grey cortex sign, endosteal callus, periosteal callus, and fracture line were accepted as the radiographic signs marking a stress injury to bone. In addition, cases of normal findings on plain radiography with medical advice sought for the second time on account of prolonged exercise-induced pain in the pelvic area or the lower extremities were referred for MRI examination at the main military hospital. The patients with stress fractures on MRI were included in the study. The remainder of the subjects under observation constituted controls for the stress fracture cases.

Identification of respiratory infections

Medical records for all participants covering the first six months of military service were abstracted, and physician-diagnosed respiratory tract infections (i.e., sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, and laryngitis) were recorded. As the incidence index we used the number of days of absence from duty due to respiratory tract infection.

Methods

Blood samples for determination of serum 25OHD concentration were drawn at entry into military service at the beginning of July 2002. After coagulation at room temperature for one hour, the samples were centrifuged at 2,000 g for 20 minutes at room temperature for serum separation. Then the serum samples were frozen and stored at –20 °C for later analysis. Total serum 25OHD concentrations were measured with an OCTEIA® enzyme immunoassay by IDS (Immunodiagnostic Systems). According to the manufacturer, the IDS intra- and inter-assay CVs for the assay are <8% and <10%, respectively, with a precision range of 5.3–380 nmol/l (CV <10%). In addition, the cross-reactivities for 25OHD3 and 25OHD2 are 100% and 75%, respectively. The 25OHD enzyme immunoassay correlates well with a radioimmunoassay (RIA), r2 = 0.82.

Subjects and methods (IV)

For the placebo-controlled double-blinded study, 164 young Finnish male volunteers (18–28 yrs) were recruited. They were conscripts in an infantry unit of the Finnish Defence Forces (the Pori Brigade, in south-west Finland) and were undergoing mandatory periodic military training. They represented the general conscript population of the Finnish Defence Forces. The trial was performed from October to March. Inclusion criteria for the study were lack of regular medication and passing of the initial medical examination as healthy. The exclusion criteria were the use of supplementary vitamin D, multivitamins, and cod-liver oil. In all, 164 men (of a total of 400 men entering that infantry unit) (41%) volunteered to participate and met the inclusion criteria. Then the subjects were randomly assigned to the intervention group (n = 80), receiving 10 µg of vitamin D3 (Minisun®, Verman) daily, or the control group (n = 84), receiving a placebo (Pharmia, a capsule identical in size and form to the active preparation). Computer-generated random numbers were used for random allocation. For the subjects, the conditions related to physical activity, nutrition, clothing, accommodation, and exposure to sunlight were homogeneous during the military service. The Ethics Committee of Tampere University Hospital, Finland, approved the study. It was registered in ClinicalTrials.gov as NCT00973583. Written informed consent was obtained from all voluntary participants.

Identification of respiratory infections

The primary outcome variable was the number of days of absence from duty because of acute respiratory tract infection. Self-reported symptoms of acute respiratory tract infection (cough, runny nose, sore throat, fever, or common-cold symptoms) and hospitalisation due to acute respiratory tract infection were secondary outcome variables. The symptoms were evaluated four times during

the study. Medical records for all participants covering the first six months of military service were reviewed, and any acute respiratory tract infection diagnosed (i.e., sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, and laryngitis) was recorded. All acute respiratory tract infections diagnosed were treated in the garrison hospital. Both the subjects and the personnel treating patients were blinded to treatment allocation.

Methods

After computer-generated random allocation, blood samples were drawn from 73 subjects at the start of the study (in October 2005) and again from 108 subjects in March 2006 to determine the serum 25OHD concentrations. The blood samples were coagulated at room temperature for an hour and centrifuged at 2,000 g for 20 min at room temperature for serum separation. The samples were frozen and stored at –20 ºC. For total serum 25OHD concentrations, an OCTEIA® enzyme immunoassay kit (Immunodiagnostic Systems) was used.

To determine plasma parathyroid hormone concentrations, 104 subjects were selected at random at the end of the study. Plasma parathyroid hormone concentrations were measured by electrochemiluminescence (Elecsys PTH Kit; Roche Diagnostics), and Elecsys PTH CalSet (Roche Diagnostics) was used for calibration.

Statistical analysis

In the articles: Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men and An association of serum vitamin D concentrations <40 nmol/l with acute respiratory tract infection in young Finnish men (I, III)

Serum 25OHD concentration was expressed as means ± SDs. Differences in means between independent samples were tested through the use of Student's t-test or one-way analysis of variance (ANOVA). The number of days of absence from duty due to respiratory infection was expressed as medians and as lower and upper quartiles (Q1 and Q3, respectively) because the distribution was skewed. Poisson regression analysis was used to explain this variable plus 0.5, because the variable included zeroes. Over-dispersion was estimated by the deviation divided by its df. Serum 25OHD concentrations were categorised as <40 nmol/l and ≥40 nmol/l for the regression analysis. The limit for statistical significance was set to be equal to 0.05 for a two-sided test. We used the software SAS for Windows (version 8.2, from the SAS Institute; Cary, NC, USA) for Poisson regression analysis and SPSS for Windows software (version 11.0, from the SPSS Institute; Chicago) for other data analysis.

In the article: Association between serum 25OHD concentrations and bone stress fractures in Finnish young men (II)

At stage 1 in the statistical analysis, the skew-continuous serum 25OHD values were divided into two categories on the basis of the median. The differences in serum 25OHD between the groups were tested by Pearson x2 testing. The results were corroborated by Mann-Whitney's U-test, using the original values. Student's t-test was used to test differences in age, BMI, height, weight, muscle strength, and 12-minute running between the groups. The limit for statistical significance was set at 0.05. Data analysis was performed with SPSS for Windows (version 11.0; SPSS Institute).

At stage 2 in the statistical analysis, logistic regression was applied to study the association between stress fractures and the statistically significant explanatory variables from stage 1. ORs were calculated with a 95% CI. Variables showing statistical significance in stage 1 (dichotomised serum 25OHD concentration, muscle strength, and 12 minutes' running) were included in the same forward stepwise regression model. Data analysis was performed with SPSS for Windows (version 12.0; SPSS Institute).

In the article: Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomised, double-blinded trial among young Finnish men (IV)

By recruiting only voluntary conscripts for the study (i.e., no sampling outside the target population was used), we aimed to maximise the power. The primary analysis included all randomised subjects, following the intention-to-treat principle. Mann-Whitney U-testing was used to test differences between the groups for continuous variables, and Chi-square tests were used to assess categorical data. A two-sided p value of less than 0.05 was set as the alpha criterion.

The hazard ratio (HR) was calculated by Cox's regression analysis adjusted for influenza vaccination and smoking at baseline; the first infection was used as the end point, with censoring at premature release from duty or the end of the study, after the six-month follow-up. The frequency of missing variables at the baseline varied between 2% and 6%. For smoking, it was 20%. Sixty subjects had dropped out of the study by the end point, with no specific reason given. Data analysis was performed with SPSS for Windows (version 15.0.1, from SPSS Institute; Chicago).

SUMMARY OF THE RESULTS

Improved vitamin D status after implementation of vitamin D fortification as public health policy in Finland (I)

In a before-and-after vitamin D fortification comparison study in January 2003 (n = 96) and in January 2004 (n = 100), nearly one year after national vitamin D fortification began, with a study population of 196 young Finnish men, the mean daily intake of milk and of margarine were 8 dl and 30 g, respectively. Therefore, after fortification the study subjects were ingesting, on average, 7 µg of vitamin D [(8 dl milk x 0.5 µg/dl) + (30 g margarine x 10 µg / 100 g)], daily through fortified products.

Mean serum 25OHD concentrations increased by 50% after implementation of the vitamin D fortification of dairy products, and the prevalence of vitamin D insufficiency (serum 25OHD <40 nmol/l) decreased by 50%, from 78% to 35%. Moreover, the prevalence of vitamin D deficiency (serum 25OHD <25 nmol/l) decreased from 19% to 5%.

In addition, five subjects (5%) had serum 25OHD concentration higher than 100 nmol/l (101–111 nmol/l) after fortification.

The association of low serum 25OHD concentration with stress fractures and acute respiratory tract infections in young Finnish men (II, III)

Low serum 25OHD concentration as a predisposing factor for stress fractures in 756 military recruits

In three-month follow-up of a population of 756 recruits, 22 subjects with stress fractures were identified (2.9%). The median serum 25OHD concentration for all the subjects was 75.8 nmol/l (range: 25.2–259.0 nmol/l).

The median serum 25OHD concentration in the group with stress fractures (median: 64.3, range: 40.1–159.0) was statistically significantly lower than that in the group of recruits without stress fractures (median: 76.2, range: 25.2–259.0, n = 734). Those with serum 25OHD levels below the median had a statistically significantly greater risk of stress fractures than those above the median.

The incidence of stress fractures was 11.6 (95% CI, 6.8–16.5) per 100 person-years. A developing stress injury became symptomatic, on average, 39 days (range: 4–70 days) after the start of military training. Thirteen bone stress injuries were located in the lower right limb and 17 in the left. In the 22 patients, plain radiography revealed 30 stress fractures in all, of which 13 (43%) were in the tibia, 10 (33%) in the metatarsal bones, three (10%) in the calcaneus, and two (7%) in the tarsal navicular bone. One fracture each was found in the inferior ramus and the femur.

The recruits with stress fractures had statistically significantly worse results in the 12-minute running test (mean: 2,480 vs. 2,670 m, p = 0.007) and in muscle strength (mean: 7 vs. 9, p = 0.025) than did controls. There was no statistically significant association between daily smoking (p = 0.85), BMI (p = 0.41), age (p = 0.27), height (p = 0.15), or weight (p = 0.70) and stress fractures. Moreover, in the multivariate regression model adjusted for all statistically significant variables from stage 1, the risk of stress fractures in subjects with serum 25OHD levels below the median was 3.6 (95% CI: 1.2–11.1) times the risk for those with concentrations exceeding the median level. In the multivariate analysis, the results for muscle strength or in the 12-minute running tests did not show statistically significant correlation with stress fractures.

Low serum 25OHD predicting respiratory tract infection

Eight hundred young Finnish men serving on a military base in Finland were enrolled for a study of whether an association exists between vitamin D insufficiency and acute respiratory tract infections. Their serum 25OHD concentrations were measured at the beginning of the study, in July 2002, and they were followed up for six months. The days of absence from duty due to respiratory infection were counted.

The mean serum 25OHD concentration was 80.2 ± 29.3 nmol/l (n = 756), and 25OHD concentrations <40 nmol/l were found in 3.6% of the subjects in July 2002. Subjects with 25OHD concentrations <40 nmol/l (n = 24) had statistically significantly (P = 0.004) more days of absence from duty due to respiratory infections (median: 4, Q1–Q3: 2–6) than did control subjects (n = 628) (2, 0–4, incidence rate ratio: 1.63, 95% CI: 1.15–2.24). The model was adjusted for smoking (n = 169).

A non-statistically significant association was seen between BMI and serum 25OHD concentration but no association between serum 25OHD concentration and lactose-intolerance. No differences were found in vitamin D status between subjects from southern and northern Finland, or between subjects living in urban and rural areas. Education level was not associated with vitamin D status.

Positive association of regular exercise with serum 25OHD concentrations

There was a statistically significant positive association between frequency of previous physical exercise and serum 25OHD concentration ($p = 0.004$). Subjects who exercised over five hours per week had higher serum 25OHD concentrations (85.8 ± 30.6 nmol/l) than did subjects reporting no physical exercise (71.3 ± 32.0 nmol/l). We also found a non-statistically significant association between frequency of physical exercise and number of days absent from duty due to respiratory infection ($p = 0.388$, $n = 264$).

Negative association of smoking with serum 25OHD concentrations

Serum 25OHD concentrations were statistically significantly ($P < 0.001$) lower in subjects who smoked (72.8 ± 26.6 nmol/l, $n = 192$) than in control subjects (82.9 ± 29.7 nmol/l, $n = 537$). There was also a trend toward a statistically significant association ($P = 0.065$) between smoking ($n = 169$) and number of days of absence from duty due to respiratory infection (incidence rate ratio: 1.18, 95% CI: 0.988–1.40, $n = 629$).

Vitamin D supplementation's prevention of respiratory tract infections in young Finnish men (IV)

Vitamin D supplementation with 10 µg/d and its substantial effect on vitamin D status

There was no difference in serum 25OHD concentration between the intervention (78.7 ± 14.9 nmol/l, $n = 29$) and placebo (74.4 ± 20.8 nmol/l, $n = 44$) group ($p = 0.35$) at the beginning of the study, in October 2005. Age, height, weight, and body mass index were also comparable between the groups at baseline, though both smoking and influenza vaccination were slightly more common in the placebo group. After daily supplementation with 10 µg of vitamin D3 or a placebo for six months, the mean serum 25OHD concentration was 71.6 ± 22.9 nmol/l ($n = 58$) in the intervention group and 51.3 ± 15.5 nmol/l ($n = 50$) in the placebo group ($p < 0.001$).

A slight preventive effect of vitamin D supplementation of 10 µg/d on the incidence of acute respiratory tract infections

The number of days absent from duty because of respiratory tract infection (the main outcome variable) did not differ between groups – the mean number of days of absence was 2.2 (SD: 3.2) in the intervention group and 3.0 (SD: 4.0) in

the placebo group (p = 0.096). However, there was an effect during the first six weeks of the study, with a mean of 0.7 (SD: 2.1) days of absence in the intervention and of 1.4 (SD: 2.6) days' absence in the placebo group (p = 0.060). After the first six weeks, there tended to be no difference between the groups.

The proportion of subjects remaining healthy throughout the six-month study period was greater in the intervention group (51.3%, n = 41) than in the placebo group (35.7%, n = 30, p = 0.045). Moreover, in a Cox regression analysis with adjustment for smoking and influenza vaccination, the adjusted hazard ratio for absence from duty due to respiratory tract infection was lower in the intervention group (HR 0.71, 95% CI: 0.43–1.15), and the number needed to treat calculated from the proportion of men without any days absent from duty was 6.4 (95% CI: 3–257).

From the questionnaire, self-reported cough (65% in the intervention group vs. 57% in the placebo group, p = 0.30), runny nose (74% vs. 75%, p = 0.86), sore throat (48% vs. 45%, p = 0.77), fever (31% vs. 38%, p = 0.36), and symptoms of the common cold (56% vs. 52%, p = 0.40) did not differ between the groups.

The mean number of days of treatment in hospital was 0.31 (SD: 1.21) per subject in the intervention group and 0.90 (SD: 2.22) in the placebo group (p = 0.06). Two subjects in the intervention group reported nausea, stomach ache, and diarrhoea during the study. In addition, one subject in the placebo group dropped out of the study because of a facial rash.

Vitamin D supplementation with 10 µg/d – no effect on plasma PTH concentrations

Plasma PTH concentrations did not differ statistically significantly between the intervention 4.3 ± 1.3 ng/l (n = 58) and placebo 4.4 ± 1.4 ng/l (n = 50) group (p = 0.55).

DISCUSSION

Vitamin D insufficiency, commonplace among young Finnish men

All Finnish men are required to complete six, nine, or 12 months of military service between the ages of 18 and 29. Each year, on average, 26,500 conscripts undergo military training. As 85–90% of Finnish men serve in the military, the conscripts represent the vast majority of all young men of the same age in Finland. The study populations for these studies were composed of these healthy young men completing military service as conscripts in the Finnish Defence Forces. The population presents homogeneity with respect to age, the conditions for physical activity, nutrition, clothing, living area, and exposure to sunlight in the military environment. The homogeneity of our study setting and population offers an optimal setting for this kind of study and can be seen as a major strength of the studies.

According to our studies, the prevalence of vitamin D insufficiency, defined as serum 25OHD <40 nmol/l, in young Finnish men was only 4% in July 2002, increased to 80% in January 2003, and diminished to 35% in January 2004 on account of nationwide vitamin D fortification. Further, vitamin D deficiency (25OHD <25 nmol/l) was seen in 19% of subjects in January 2003 and in 5% in January 2004, with mean serum 25OHD levels of 34 and 50 nmol/l, respectively. The level was 80 nmol/l in July 2002. In other studies of young Finnish men, 2% had a serum 25OHD of <20 nmol/l in July 2001, 34–39% in January 2001, and 29% in January 2004 – one year after the vitamin D fortification began. The corresponding median levels of serum 25OHD were 44, 24, and 27 nmol/l, respectively (Välimäki et al. 2004; Välimäki et al. 2007).

For vitamin D determination, one must take into account that the values differ, depending on the technique used. For total serum 25OHD concentrations, we used an OCTEIA® enzyme immunoassay kit (Immunodiagnostic Systems) while in other Finnish studies described above RIA (Diasorin) or HPLC was used. In earlier evaluation of vitamin D measurement methods, HPLC was considered the gold standard of 25OHD measurement. For the IDS enzyme-linked immunoassay, there has been mixed performance, with up to 23% overestimation until 2006, that might explain the differences between our studies and Finnish studies that used other techniques (de la Hunty et al. 2010).

The present studies showed a statistically significant positive association between regular physical exercise and serum 25OHD level. The subjects exercising regularly were vitamin D sufficient in summertime (86 nmol/l). Similarly, a recent Australian study showed statistically significantly higher prevalence of vitamin D deficiency in those who were physically inactive. In that study, vitamin D deficiency was defined as serum 25OHD <50 nmol/l and found in nearly one third of Australian adults (Daly et al. 2011). All of these studies support the conclusion that regular physical exercise is related to a greater amount of time spent outdoors and is a strong determinant of improved vitamin D status.

Obesity is thought to be a risk factor for vitamin D insufficiency. This is explained by reduced outdoor physical activity; by sequestration of fat-soluble serum 25OHD in adipose tissue, from which it cannot be easily released; and by increased need for vitamin D in the obese because of stronger bones supporting more weight (Renzaho et al. 2011). Again, in a large nationwide Australian study, serum 25OHD levels were statistically significantly lower in obese men and women than among those of normal weight (Daly et al. 2011). Further, vitamin D levels have been shown in another study to be substantially lower in obese people than in those of normal weight after equal doses of UVB (Wortsman et al. 2000). However, the results of other studies are somewhat contradictory. In our study, there was only a non-statistically significant association between BMI and serum 25OHD concentration. The relationship between serum 25OHD and BMI remains unclear and requires further investigation, as ethnicity, gender, and age may play a crucial role here (Renzaho et al. 2011).

The present studies showed no statistically significant differences in vitamin D status between subjects from southern and northern Finland, between subjects of different education levels, or between subjects living in urban vs. rural areas. In contrast, in a recent Australian study the highest rates of vitamin D deficiency (serum 25OHD <50 nmol/l) were found among educated subjects and at a latitude above 35° S in wintertime (Daly et al. 2011). Our studies were expected to show differences between subjects from northern and southern Finland due to the geographical location of the country, extending from roughly 60° to 70° N.

The present studies showed a statistically significant negative association between smoking and serum 25OHD. Similarly, high prevalence of low 25OHD levels was found in continuous smokers with established mild or moderate COPD in a recent study (Kunisaki et al. 2011). The relation between smoking and vitamin D status is still unclear but might be explained by the effects of nicotine metabolites on hepatic CYP27A1 activity.

The results of the present studies show that, in terms of adequacy of sunlight exposure, cutaneous synthesis of vitamin D is sufficient in summer in young Finnish men and was even improved with the national policy of vitamin D fortification of dairy products. However, vitamin D insufficiency is likely in young Finnish men without vitamin D supplementation during winter. The findings are recognised as having significant public health implications globally.

Vitamin D fortification of milk products – improved vitamin D status but no resolution of hypovitaminosis in young Finnish men

National vitamin D fortification started in February 2003 on the recommendation of the Ministry of Social Affairs and Health in Finland. It covers liquid milk products and margarines. In practice, all manufacturers have complied with the recommendation. In our study, vitamin D status was determined before and after vitamin D fortification (January 2003 and January 2004), both times during army service. In the study, nationwide fortification substantially improved the vitamin D status of young Finnish men but did not resolve hypovitaminosis in young Finnish men, as 35% remained vitamin D insufficient. Mean serum 25OHD concentrations increased by 50%, from 33.5 to 50.2 nmol/l. In another study, with 65 subjects, the vitamin D fortification increased mean serum 25OHD concentrations to a limited extent: by 20% in young Finnish men. In that study, vitamin D status was determined first in January 2001 and then in January 2004, a few years after army service. The use of milk products was different during the army service, and in the sub-population with ≥7.2 dl of milk daily there was actually a >40% improvement in vitamin D status (Välimäki et al. 2007). In our study, given the average daily intake of milk and margarine in the Finnish Defence Forces, the subjects ingested 7 µg more vitamin D after the fortification than before, corresponding to the target level set by the Finnish National Nutrition Board. In earlier studies, every microgram of vitamin D taken daily has increased serum 25OHD by 0.6–2.2 nmol/l. In conclusion, national vitamin D fortification increased vitamin D status as expected in our study (Byrne et al. 1995; Heaney et al. 2003; Heaney 2007).

National fortification of dairy products might include a risk of a possibility of overdose in some individuals. Hypervitaminosis D has been reported rarely and can occur only with supplementation as skin regulates the production of vitamin D by itself. The toxicity of vitamin D is due to hypercalcemia and hyperphosphatemia. Secondary to hypercalcemia hypercalciuria, kidney failure, nausea, vomiting, confusion and weakness has been seen in hypervitaminosis D patients. (Holick 2007) The toxicity may occur in serum 25OHD levels >375 nmol/l. Case reports of hypervitaminosis D include supplement use that contained much more vitamin D than label indicated. In one case, leading to hypervitaminosis D, a patient had consumed 5000 µg of vitamin D3 daily for two months because of an error in product formulation (Klontz et al. 2007). However, in another case report the usage 3750 µg of vitamin D2 daily for many years did not cause hypervitaminosis D symptoms (Jacobsen et al. 2011). Though information on symptoms was not systematically collected via questionnaires and we did not have information on vitamin D intake at individual level, no clinical signs indicating toxicity were seen in our study. Only 5% of the subjects in January 2004 and none of the subjects in January 2003 had a serum 25OHD concentration of >100 nmol/l.

The effects of the vitamin D fortification on vitamin D status in young Finnish men (1 µg / 100 ml in liquid milk products and 20 µg / 100 g of fat spreads) that began in April 2010 after the recommendation of the Finnish National Nutrition Council still needs to be clarified (Pietinen et al. 2010). Further, daily vitamin D supplementation of 10 µg for children <2 years, pregnant and breast-feeding women, 7.5 µg for 2–18-year-olds, and 20 µg for those over 60 years has been recommended by the Council since January 2011 in Finland. The long-term effects on health remain to be demonstrated.

The association of low serum 25OHD concentration with stress fractures in young Finnish men

The study demonstrates that the subjects with stress fractures had substantially lower serum 25OHD concentrations (64 nmol/l) than did controls (76 nmol/l). The study showed that in the group with a serum 25OHD level less than the median (76 nmol/l), the risk of stress fractures was 3.6 times that of 1 in the control group. In addition, those with stress fractures had substantially poorer results in the 12-minute running test and for muscle strength than controls did in the univariate analysis. No statistically significant association was found between smoking, BMI, age, height, or weight with stress fractures.

In the multivariate analysis, the results for muscle strength or 12-minute running tests were not statistically significantly associated with stress fractures. However, there was a statistically significant association between a serum 25OHD level below the median and stress fractures also in the multivariate analysis. It is known that vitamin D increases intestinal calcium absorption and its renal reabsorption, maintaining a reasonable amount of calcium in circulation, especially for bone mineralisation (Utiger 1998). Vitamin D is also known to decrease PTH secretion and enhance both osteoblast and osteoclast differentiation (Riggs 1997; Holick 2003). Vitamin D has a major enhancing effect on peak bone mass too (Vieth 1999; Välimäki et al. 2004). In a Finnish study, stress fractures were associated with higher PTH levels but not with lower 25OHD levels (Välimäki et al. 2005). Earlier, a correlation between low BMD and stress fractures was demonstrated in military recruits (Pouilles et al. 1989). Furthermore, in the Israeli Defence Forces study, serum 25OHD was substantially lower among patients with high-grade stress fractures than among the controls (Givon et al. 2000). All of these findings strongly suggest that inadequate vitamin D is a risk factor for stress fractures in military recruits.

A negative correlation between PTH and 25OHD levels has been demonstrated in earlier studies: PTH starts to increase at serum 25OHD levels below 78–100 nmol/l (Chapuy et al. 1997; McKenna et al. 1998). Further, increased calcium absorption in the intestine has been demonstrated following a rise in serum 25OHD concentration from 50 to 86 nmol/l (Heaney et al. 2003). Interestingly, in our study recruits with serum 25OHD of <76 nmol/l exhibited substantially more stress fractures than did those with higher concentrations. The

results from our study suggest that the risk of stress fractures is diminished when serum 25OHD levels exceed 78–100 nmol/l throughout the year.

A recent vitamin D supplementation RCT with 2 g calcium and 20 µg vitamin D daily for eight weeks demonstrated a 20% decrease in the incidence of stress fractures (Bouillon 2008; Lappe et al. 2008). The double risk of stress fractures of the tibia and fibula was demonstrated with serum 25OHD <50 nmol/l as compared to those with >100 nmol/l in female US Navy recruits. In the study, an inverse dose–response gradient between serum 25OHD and risk of stress fractures was demonstrated also (Burgi et al. 2011). Moreover, a recent study found two polymorphisms in the VDR to increase the risk of stress fractures independently, suggesting screening for VDR polymorphisms as a tool for identifying individuals at increased risk of stress fractures during physical and/or military training (Chatzipapas et al. 2009; McClung et al. 2010). The findings from these studies support the findings in our study.

Vitamin D insufficiency as a predictor of higher risk of acute respiratory tract infection in young Finnish men

The cohort study showed a statistically significant negative association of serum 25OHD concentration with the number of days of absence from duty due to physician-diagnosed respiratory tract infection. Because living quarters are close for recruits, respiratory tract infections are commonplace and easily transmitted in garrisons, offering ideal circumstances for this kind of study. Therefore, it is likely that low vitamin D status upon initial entry into military service contributes to proneness to respiratory tract infections.

In addition to the homogeneity of the conditions during the study for all subjects, enhancing the results' validity, the study has other strengths. Firstly, the accuracy of the number of physician-diagnosed respiratory infections identified with respect to absence from duty can be considered a positive element. Secondly, a statistically significant effect on absence from duty due to infectious diseases was found despite the fact that a relatively small number of vitamin D insufficient young men was found at the beginning of the study, in July 2002. Thirdly, the effect of smoking on respiratory infections was adjusted for the study.

Persistence of vitamin D sufficiency throughout the study was not determined; vitamin D status was assessed only at the beginning of the study, in July 2002. This can be seen as a possible weakness of the study. In addition, vitamin D was statistically significantly correlated with the amount of previous physical exercise but we found only a non-statistically significant association between frequent physical exercise and days of absence from duty due to respiratory infection, not confounding the results.

Recent evidence shows that vitamin D upregulates the expression of antimicrobial peptides that proved to inhibit invasive pneumococcal disease, meningococcal disease, and group A streptococcal disease in an earlier study

(Vlaminckx et al. 2005). Further, beta defensins that belong to the AMP family were shown to inhibit the growth of otitis media pathogens (Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae) in another study (Lee et al. 2004). A polymorphism in the VDR gene was also positively associated with respiratory-syncytial-virus-related disease in recent studies, with both South African and Dutch children (Kresfelder et al. 2011). The findings are consistent with the idea from other studies that secreted antimicrobial peptides are dependent on vitamin D and as a part of innate immunity constitute the first line of defence protecting host mucosal surfaces' against microbes (Hewison 2011; Laaksi 2011). The findings support also the finding of our cohort study that low vitamin D levels pose a risk for a respiratory tract infection.

Adequate vitamin D status was proposed to protect against influenza in a cohort study with 7,000 participants aged over 45 years. In this study, the prevalence of respiratory infections showed a strong seasonal pattern inversely related to 25OHD concentrations. With adjustment for adiposity, lifestyle, and socio-economic factors, each 10 nmol/l rise in serum 25OHD concentration was associated with a 7% lower risk of infection in the study. Vitamin D status seems to have a linear relationship with respiratory infections (Berry et al. 2011). Further, in a secondary analysis of the Third National Health and Nutrition Examination Survey (1988–1994), with almost 19,000 participants aged above 12 years, serum 25OHD concentrations were inversely associated with recent URTI. The analysis took into consideration season, BMI, smoking, asthma, and COPD, with URTIs reported by 24% of subjects with serum 25OHD <25 nmol/l, by 20% with 25–75 nmol/l, and by 17% with >75 nmol/l (Ginde et al. 2009). These findings are consistent with the results of our study.

Possible preventive effect of vitamin D supplementation on acute respiratory tract infection during military service

The present RCT with 164 young Finnish men provided some evidence of a preventive effect of vitamin D supplementation against respiratory tract infection. Firstly, the proportion of the subjects who remained healthy throughout the study period was substantially greater in the intervention than in the placebo group. Secondly, the adjusted HR for absence from duty due to respiratory tract infection was lower in the intervention than in the control group. Thirdly, the number needed to treat calculated from the proportion of men without any days absent from duty was as low as 6.4. In addition, an effect was found during the first six weeks of the study, with fewer days of absence in the intervention than in the placebo group. Also, a trend in which the mean number of days of treatment in hospital was diminished by 10 µg of vitamin D supplementation daily was found in the present study.

In a 5 month RCT conducted in Antarctica during winter when ultraviolet B radiation levels are essentially zero showed that vitamin D supplementation with 50 µg/d increased serum 25OHD level to 71 nmol/l. On the other hand in the 10

µg/d subgroup an increase up to 57 nmol/l was seen. (Smith et al. 2009) In contrast, a study in submariners showed no increase in serum 25OHD with 10 µg/d of vitamin D supplementation (Duplessis et al. 2005). Importantly, a daily 10 µg of vitamin D during winter was able to keep vitamin D status almost adequate (25OHD >75 nmol/l) while levels in subjects without supplementation remained mainly insufficient (25OHD <50 nmol/l) in our study. Again, the technique used for determination of vitamin D levels has to be taken into account.

The study had some limitations. Our main outcome variable, the number of days' absence from duty due to respiratory tract infection, did not differ between the intervention and control group. Further, self-reported symptoms of respiratory tract infection (cough, runny nose, sore throat, fever, and symptoms of the common cold) did not differ between the groups. There was also a very wide 95% CI, 3–257, in the number needed to treat calculated from the proportion of men without any days absent from duty. Because of the number of subjects who withdrew from the study, its statistical power remains limited.

The completeness of the outcome data related to respiratory infections identified and the amount of days of absence from duty is a strength of the study. Interestingly, if no subjects had withdrawn from the study, a statistically significant preventive effect of 10 µg daily vitamin D supplementation on respiratory tract infections would have been shown. On the other hand, when we used a one-sided P value in the analysis, the difference in days absent from duty between the trial arms was statistically significant.

In the present study, 10 µg of vitamin D daily did not have a statistically significant effect on the study subjects' PTH concentrations. However, in another six-month Finnish RCT, elevation of PTH concentration was inhibited by 20 µg of vitamin D daily. It is probable that the dose used in our study was not enough and that an effect on PTH concentration would have been seen with a higher supplementation dose (Viljakainen et al. 2008).

In an RCT with >200 healthy post-menopausal African American women who were given 20 µg of vitamin D daily or a placebo for two years, a lower rate of self-reported upper respiratory infection or influenza was observed in the intervention arm as compared to the placebo group. After an increase in vitamin D dosage from 20 to 50 µg of vitamin D daily for one year, the effect was even greater (Aloia et al. 2007; Li-Ng et al. 2009). This finding supports the results of our study.

Another RCT failed to show any statistically significant difference between the vitamin D and placebo group in the secondary end point of self-reported infection rate. In that study, 3,500 elderly subjects were given 20 µg of vitamin D or a placebo for over two years. However, because of the poor compliance observed, the results are complicated – only half of the subjects remained compliant with use of the study medication at two years' follow-up. In addition, a relatively inadequate increase, from 38 to 63 nmol/l, in serum 25OHD levels was shown in the intervention group after vitamin D therapy (Avenell et al. 2007).

Similarly, a recent RCT with 162 healthy adults given 50 µg of vitamin D or a placebo daily for 12 weeks during the winter and spring months showed no benefit with respect to the incidence or severity of upper respiratory infection symptoms. In the study, appropriate increase in serum 25OHD levels in the intervention group from 62 to 89 nmol/l at 12 weeks was noted. However, a statistical tendency to favour the vitamin D group was seen (Li-Ng et al. 2009).

CONCLUSIONS

Based on our study vitamin D insufficiency was found in relatively low proportion of the subjects during summer. However, the study shows that vitamin D insufficiency is commonplace in Finland during winter even after vitamin D fortification, with 35% of young Finnish men being vitamin D insufficient in January 2004. Furthermore, it is likely that vitamin D levels reach their lowest in late winter and vitamin D insufficiency can then be found in substantially more than 35% of young men. It is notable that the studies were carried out before the Finnish National Nutrition Board had given new recommendation of vitamin D fortification in 2010. In order to maintain an adequate level of vitamin D, i.e. serum 25OHD >75 nmol/l, throughout the year young Finnish men will need an additional supplementation with 20 µg of vitamin D daily during winter.

In conclusion, because of vitamin D inadequacy, there seems to be a strong likelihood of considerable risk of stress fractures among young men during military service. A good target for prevention of stress fractures might be a serum 25OHD of >75 nmol/l throughout the year in young Finnish men. Interestingly our RCT with 10 µg/d of vitamin D found no effect on plasma PTH levels in the young Finnish men. Given this and earlier intervention studies' results on improving vitamin D status, an adequate serum 25OHD level (>75 nmol/l) will be achieved through 20 µg of vitamin D supplementation daily during winter in Finland (Byrne et al. 1995; Heaney et al. 2003; Heaney 2007; Burgi et al. 2011).

The cohort study performed that low vitamin D status at entry into military service and subsequent respiratory infections have a clear statistically significant relationship. In order to prevent acute respiratory tract infections in young Finnish men, at least during military service, an additional supplementation with 20 µg of vitamin D daily will be needed during winter. Clarification of the role of vitamin D in relation to acute respiratory tract infections represents a high priority for future research.

The RCT conducted for this research provided some evidence of vitamin D supplementation acting against acute respiratory infections in young Finnish men during winter in military service. However, results from other RCTs of vitamin D for the treatment and prevention of viral upper respiratory tract infections in adult populations are somewhat controversial and suggest that a larger sample, adequate vitamin D repletion, and a longer period may be beneficial in the design of future studies, to maximise immunomodulatory effects. Further, the results of the present RCT indicate that additional supplementation, to 20 µg of

vitamin D daily during winter may have the preventive effect on acute respiratory tract infections in young Finnish men during military service.

ACKNOWLEDGEMENTS

This study was carried out at the Department of Cell Biology, Medical School, University of Tampere and at the Centre of Military Medicine, Finnish Defence Forces, Finland.

I am grateful to my supervisor Professor Timo Ylikomi M.D., Ph.D. for patient guidance, encouragement and positive support during these years. His enthusiasm for science and scientific visions were essential for the study. I appreciate that he allowed me to participate in many kind of research projects that have taught me scientific thinking throughout these years.

I am grateful to my supervisor Docent Harri Pihlajamäki, M.D., Ph.D. for his patient guidance, continuous encouragement and warm support during the study. His practical advice and experience in the field of science were of greatest importance for the study. I thank him for the possibility to scientific co-operation with his group in the Centre of Military Medicine. That was essential for carrying out the present study.

I am grateful to official reviewers of the thesis: Professor Ilari Paakkari M.D., Ph.D. and Professor Tomi-Pekka Tuomainen M.D., Ph.D. for careful revision, advice and valuable comments.

I am grateful to the members of follow-up group. I thank Professor Pentti Tuohimaa, M.D., Ph.D. for valuable advice and help during these years. I thank Heimo Syvälä, Ph.D. for practical advice, encouragement and help.

I am grateful to Professor Anssi Auvinen, M.D., Ph.D., for the statistical advice and help. I am grateful to Docent Ville Mattila, M.D., Ph.D. and Riina Lemponen, M.Sc. for statistical advice. I thank Jaakko Pitkäjärvi, M.D. for help.

I am grateful to Professor Seppo parkkila, M.D., Ph.D., Docent Paula Kujala, M.D., Ph.D., Professor Markku Miettinen M.D., Ph.D., Merja Bläuer, Ph.D. and Yan-Ru Lou, Ph.D. for advice and encouragement.

I am grateful to Juha-Petri Ruohola, M.D., Ph.D. for the scientific co-operation and friendship during these years.

I thank Anu Planting for careful language revision of the thesis.

I thank Hilkka Mäkinen and Arja Ahola for excellent technical assistance. I owe my thanks to Riina Sarkanen, Sami Purmonen, Pasi Pennanen, Tommi Manninen, Marianne Kuuslahti, Tytti Ahola, Merja Ahonen, Annika Hakamäki, Susanna Miettinen, Ulla Järvelin, Mika Perttu, Teemu Murtola, Ya-Hua Zhuang, Allan Kalueff, Nanna Sarvilinna, Katja Ahtiainen, Tiina Mäkelä, Lars Wichmann, Niina Ikonen, Ulla Jukarainen, Taina Eskola, Janette Hinkka, Mirja Hyppönen, Tarja Arvela, Marja Eloranta, Seija Kivimäki, Leo Syrjänen and Maarit Pehkonen for the encouragement and help during these years.

I owe my thanks to Aulikki for looking after our children so many times during these years. I also thank Antti, Lissu, Ilmo, Emppu and Sanna for support and friendship.

I thank Mikko and Jaana for practical help and friendship.

I am deeply thankful to my loving parents Seppo and Riitta for the never-ending support and encouragement through these years. I thank my father for long discussions and continuous support that have been invaluable. I thank my mother for taking care of our kids when I needed time for the study. I also thank Ansku, Juha, Iku and Arto for support and encouragement.

My deepest thanks belong to my wife Leena and to my lovely children Iida, Akseli and Lotta. I love you.

The study was financially supported by grants from the Medical Research Fund of Tampere University Hospital, Finnish Cultural Foundation and Scientific Committee of National Defence Forces, Finland.

REFERENCES

Adams, J. S. and M. Hewison 2010. Update in vitamin D. J Clin Endocrinol Metab 95 (2): 471-8.

Aloia, J. F., D. G. Chen and H. Chen 2010. The 25(OH)D/PTH threshold in black women. J Clin Endocrinol Metab 95 (11): 5069-73.

Aloia, J. F., M. Feuerman and J. K. Yeh 2006. Reference range for serum parathyroid hormone. Endocr Pract 12 (2): 137-44.

Aloia, J. F. and M. Li-Ng 2007. Re: epidemic influenza and vitamin D. Epidemiol Infect 135 (7): 1095-6; author reply 1097-8.

Altarac, M., J. W. Gardner, R. M. Popovich, R. Potter, J. J. Knapik and B. H. Jones 2000. Cigarette smoking and exercise-related injuries among young men and women. Am J Prev Med 18 (3 Suppl): 96-102.

Anderson, M. W. and A. Greenspan 1996. Stress fractures. Radiology 199 (1): 1-12.

Armas, L. A., B. W. Hollis and R. P. Heaney 2004. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab 89 (11): 5387-91.

Avenell, A., J. A. Cook, G. S. Maclennan and G. C. Macpherson 2007. Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). Age Ageing 36 (5): 574-7.

Baeksgaard, L., K. P. Andersen and L. Hyldstrup 1998. Calcium and vitamin D supplementation increases spinal BMD in healthy, postmenopausal women. Osteoporos Int 8 (3): 255-60.

Beck, T. J., C. B. Ruff, R. A. Shaffer, K. Betsinger, D. W. Trone and S. K. Brodine 2000. Stress fracture in military recruits: gender differences in muscle and bone susceptibility factors. Bone 27 (3): 437-44.

Berry, D. J., K. Hesketh, C. Power and E. Hyppönen 2011. Vitamin D status has a linear association with seasonal infections and lung function in British adults. Br J Nutr 1-8.

Bikle, D. 2008. Nonclassic Actions of Vitamin D. J Clin Endocrinol Metab

Bischoff-Ferrari, H. A., T. Dietrich, E. J. Orav and B. Dawson-Hughes 2004. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 116 (9): 634-9.

Bouillon, R. 2008. How effective is nutritional supplementation for the prevention of stress fractures in female military recruits? Nat Clin Pract Endocrinol Metab 4 (9): 486-7.

Bouillon, R., G. Carmeliet, E. Daci, S. Segaert and A. Verstuyf 1998. Vitamin D metabolism and action. Osteoporos Int 8 Suppl 2 S13-9.

Brandi, M. L. 2009. Microarchitecture, the key to bone quality. Rheumatology (Oxford) 48 Suppl 4 iv3-8.

Brehm, J. M., B. Schuemann, A. L. Fuhlbrigge, B. W. Hollis, R. C. Strunk, R. S. Zeiger, S. T. Weiss and A. A. Litonjua 2010. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol 126 (1): 52-8 e5.

Burgi, A. A., E. D. Gorham, C. F. Garland, S. B. Mohr, F. C. Garland, K. Zeng, K. Thompson and J. M. Lappe 2011. High serum 25-hydroxyvitamin D is associated with a low incidence of stress fractures. J Bone Miner Res 26 (10): 2371-7.

Byrne, P. M., R. Freaney and M. J. McKenna 1995. Vitamin D supplementation in the elderly: review of safety and effectiveness of different regimes. Calcif Tissue Int 56 (6): 518-20.

Cannell, J. J., R. Vieth, J. C. Umhau, M. F. Holick, W. B. Grant, S. Madronich, C. F. Garland and E. Giovannucci 2006. Epidemic influenza and vitamin D. Epidemiol Infect 134 (6): 1129-40.

Chapuy, M. C., P. Preziosi, M. Maamer, S. Arnaud, P. Galan, S. Hercberg and P. J. Meunier 1997. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 7 (5): 439-43.

Chatzipapas, C., S. Boikos, G. I. Drosos, K. Kazakos, G. Tripsianis, A. Serbis, S. Stergiopoulos, C. Tilkeridis, D. A. Verettas and C. A. Stratakis 2009. Polymorphisms of the vitamin D receptor gene and stress fractures. Horm Metab Res 41 (8): 635-40.

Christakos, S. and H. F. DeLuca 2011. Minireview: Vitamin D: is there a role in extraskeletal health? Endocrinology 152 (8): 2930-6.

Cline, A. D., G. R. Jansen and C. L. Melby 1998. Stress fractures in female army recruits: implications of bone density, calcium intake, and exercise. J Am Coll Nutr 17 (2): 128-35.

Compston, J. E. 1998. Vitamin D deficiency: time for action. Evidence supports routine supplementation for elderly people and others at risk. Bmj 317 (7171): 1466-7.

Correale, J., M. C. Ysrraelit and M. I. Gaitan 2009. Immunomodulatory effects of Vitamin D in multiple sclerosis. Brain 132 (Pt 5): 1146-60.

Cowan, D. N., B. H. Jones, P. N. Frykman, D. W. Polly, Jr., E. A. Harman, R. M. Rosenstein and M. T. Rosenstein 1996. Lower limb morphology and risk of overuse injury among male infantry trainees. Med Sci Sports Exerc 28 (8): 945-52.

Daffner, R. H. and H. Pavlov 1992. Stress fractures: current concepts. AJR Am J Roentgenol 159 (2): 245-52.

Daly, R. M., C. Gagnon, Z. X. Lu, D. J. Magliano, D. W. Dunstan, K. A. Sikaris, P. Z. Zimmet, P. R. Ebeling and J. E. Shaw 2011. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: A national, population-based study. Clin Endocrinol (Oxf)

Dawson-Hughes, B., S. Harris, C. Kramich, G. Dallal and H. M. Rasmussen 1993. Calcium retention and hormone levels in black and white women on high- and low-calcium diets. J Bone Miner Res 8 (7): 779-87.

Dawson-Hughes, B., R. P. Heaney, M. F. Holick, P. Lips, P. J. Meunier and R. Vieth 2005. Estimates of optimal vitamin D status. Osteoporos Int 16 (7): 713-6.

de la Hunty, A., A. M. Wallace, S. Gibson, H. Viljakainen, C. Lamberg-Allardt and M. Ashwell 2010. UK Food Standards Agency Workshop Consensus Report: the choice of method for measuring 25-hydroxyvitamin D to estimate vitamin D status for the UK National Diet and Nutrition Survey. Br J Nutr 104 (4): 612-9.

Duplessis, C. A., E. B. Harris, D. E. Watenpaugh and W. G. Horn 2005. Vitamin D supplementation in underway submariners. Aviat Space Environ Med 76 (6): 569-75.

Gardner, L. I., Jr., J. E. Dziados, B. H. Jones, J. F. Brundage, J. M. Harris, R. Sullivan and P. Gill 1988. Prevention of lower extremity stress fractures: a controlled trial of a shock absorbent insole. Am J Public Health 78 (12): 1563-7.

Ginde, A. A., J. M. Mansbach and C. A. Camargo, Jr. 2009. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 169 (4): 384-90.

Givon, U., E. Friedman, A. Reiner, I. Vered, A. Finestone and J. Shemer 2000. Stress fractures in the Israeli defense forces from 1995 to 1996. Clin Orthop Relat Res (373): 227-32.

Goltzman, D. 2010. Vitamin D action : Lessons learned from genetic mouse models. Ann N Y Acad Sci 1192 (1): 145-52.

Guryev, O., R. A. Carvalho, S. Usanov, A. Gilep and R. W. Estabrook 2003. A pathway for the metabolism of vitamin D3: unique hydroxylated metabolites formed during catalysis with cytochrome P450scc (CYP11A1). Proc Natl Acad Sci U S A 100 (25): 14754-9.

Hansdottir, S. and M. M. Monick 2011. Vitamin D effects on lung immunity and respiratory diseases. Vitam Horm 86 217-37.

Hansdottir, S., M. M. Monick, S. L. Hinde, N. Lovan, D. C. Look and G. W. Hunninghake 2008. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 181 (10): 7090-9.

Harkness, L. and B. Cromer 2005. Low levels of 25-hydroxy vitamin D are associated with elevated parathyroid hormone in healthy adolescent females. Osteoporos Int 16 (1): 109-13.

Heaney, R. P. 2007. Vitamin D endocrine physiology. J Bone Miner Res 22 Suppl 2 V25-7.

Heaney, R. P., K. M. Davies, T. C. Chen, M. F. Holick and M. J. Barger-Lux 2003. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 77 (1): 204-10.

Heaney, R. P., M. S. Dowell, C. A. Hale and A. Bendich 2003. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 22 (2): 142-6.

Heaney, R. P. and C. M. Weaver 2005. Newer perspectives on calcium nutrition and bone quality. J Am Coll Nutr 24 (6 Suppl): 574S-81S.

Hewison, M. 2011. Vitamin D and innate and adaptive immunity. Vitam Horm 86 23-62.

Hewison, M., D. Zehnder, R. Bland and P. M. Stewart 2000. 1alpha-Hydroxylase and the action of vitamin D. J Mol Endocrinol 25 (2): 141-8.

Holick, M. F. 1994. McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. Am J Clin Nutr 60 (4): 619-30.

Holick, M. F. 2003. Vitamin D: A millenium perspective. J Cell Biochem 88 (2): 296-307.

Holick, M. F. 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 80 (6 Suppl): 1678S-88S.

Holick, M. F. 2007. Vitamin D deficiency. N Engl J Med 357 (3): 266-81.

Holick, M. F., N. C. Binkley, H. A. Bischoff-Ferrari, C. M. Gordon, D. A. Hanley, R. P. Heaney, M. H. Murad and C. M. Weaver 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96 (7): 1911-30.

Holick, M. F., J. A. MacLaughlin, M. B. Clark, S. A. Holick, J. T. Potts, Jr., R. R. Anderson, I. H. Blank, J. A. Parrish and P. Elias 1980. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science 210 (4466): 203-5.

Holick, M. F., J. A. MacLaughlin and S. H. Doppelt 1981. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. Science 211 (4482): 590-3.

Holick, M. F., L. Y. Matsuoka and J. Wortsman 1989. Age, vitamin D, and solar ultraviolet. Lancet 2 (8671): 1104-5.

Holick, M. F., N. M. Richtand, S. C. McNeill, S. A. Holick, J. E. Frommer, J. W. Henley and J. T. Potts, Jr. 1979. Isolation and identification of previtamin

D3 from the skin of rats exposed to ultraviolet irradiation. Biochemistry 18 (6): 1003-8.

Holick, M. F., M. Uskokovic, J. W. Henley, J. MacLaughlin, S. A. Holick and J. T. Potts, Jr. 1980. The photoproduction of 1 alpha,25-dihydroxyvitamin D3 in skin: an approach to the therapy of vitamin-D-resistant syndromes. N Engl J Med 303 (7): 349-54.

Houghton, L. A. and R. Vieth 2006. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 84 (4): 694-7.

Hunt, C. D. and L. K. Johnson 2007. Calcium requirements: new estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. Am J Clin Nutr 86 (4): 1054-63.

Hyppönen, E., E. Laara, A. Reunanen, M. R. Järvelin and S. M. Virtanen 2001. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 358 (9292): 1500-3.

Islam, M. Z., H. T. Viljakainen, M. U. Kärkkäinen, E. Saarnio, K. Laitinen and C. Lamberg-Allardt 2012. Prevalence of vitamin D deficiency and secondary hyperparathyroidism during winter in pre-menopausal Bangladeshi and Somali immigrant and ethnic Finnish women: associations with forearm bone mineral density. Br J Nutr 1-7.

Jacobsen, R. B., B. W. Hronek, G. A. Schmidt and M. L. Schilling 2011. Hypervitaminosis D associated with a vitamin D dispensing error. Ann Pharmacother 45 (10): e52.

Jones, B. H., J. M. Harris, T. N. Vinh and C. Rubin 1989. Exercise-induced stress fractures and stress reactions of bone: epidemiology, etiology, and classification. Exerc Sport Sci Rev 17 379-422.

Jones, B. H., S. B. Thacker, J. Gilchrist, C. D. Kimsey, Jr. and D. M. Sosin 2002. Prevention of lower extremity stress fractures in athletes and soldiers: a systematic review. Epidemiol Rev 24 (2): 228-47.

Jones, G. 2008. Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr 88 (2): 582S-586S.

Jörgensen, C., M. A. Gall, A. Schmedes, L. Tarnow, H. H. Parving and P. Rossing 2010. Vitamin D levels and mortality in type 2 diabetes. Diabetes Care 33 (10): 2238-43.

Kato, S. 2000. The function of vitamin D receptor in vitamin D action. J Biochem 127 (5): 717-22.

Kiuru, M. J., H. K. Pihlajamäki and J. A. Ahovuo 2004. Bone stress injuries. Acta Radiol 45 (3): 317-26.

Klontz, K. C. and D. W. Acheson 2007. Dietary supplement-induced vitamin D intoxication. N Engl J Med 357 (3): 308-9.

Krall, E. A., N. Sahyoun, S. Tannenbaum, G. E. Dallal and B. Dawson-Hughes 1989. Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. N Engl J Med 321 (26): 1777-83.

Kresfelder, T. L., R. Janssen, L. Bont and M. Venter 2011. Confirmation of an association between single nucleotide polymorphisms in the VDR gene with respiratory syncytial virus related disease in South African Children. J Med Virol 83 (10): 1834-40.

Kunisaki, K. M., D. E. Niewoehner, R. J. Singh and J. E. Connett 2011. Vitamin D status and longitudinal lung function decline in the Lung Health Study. Eur Respir J 37 (2): 238-43.

Laaksi, I. 2011. Vitamin D and respiratory infection in adults. Proc Nutr Soc 1-8.

Lamberg-Allardt, C. J., T. A. Outila, M. U. Karkkainen, H. J. Rita and L. M. Valsta 2001. Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? J Bone Miner Res 16 (11): 2066-73.

Lappe, J., D. Cullen, G. Haynatzki, R. Recker, R. Ahlf and K. Thompson 2008. Calcium and vitamin d supplementation decreases incidence of stress fractures in female navy recruits. J Bone Miner Res 23 (5): 741-9.

Laube, D. M., S. Yim, L. K. Ryan, K. O. Kisich and G. Diamond 2006. Antimicrobial peptides in the airway. Curr Top Microbiol Immunol 306 153-82.

LeBoff, M. S., L. Kohlmeier, S. Hurwitz, J. Franklin, J. Wright and J. Glowacki 1999. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. Jama 281 (16): 1505-11.

Lee, D. M., A. Tajar, T. W. O'Neill, D. B. O'Connor, G. Bartfai, S. Boonen, R. Bouillon, F. F. Casanueva, J. D. Finn, G. Forti, A. Giwercman, T. S. Han, I. T. Huhtaniemi, K. Kula, M. E. Lean, M. Punab, A. J. Silman, D. Vanderschueren, F. C. Wu and N. Pendleton 2011. Lower vitamin D levels are associated with depression among community-dwelling European men. J Psychopharmacol

Lee, H. Y., A. Andalibi, P. Webster, S. K. Moon, K. Teufert, S. H. Kang, J. D. Li, M. Nagura, T. Ganz and D. J. Lim 2004. Antimicrobial activity of innate immune molecules against Streptococcus pneumoniae, Moraxella catarrhalis and nontypeable Haemophilus influenzae. BMC Infect Dis 4 12.

Lehmann, B. and M. Meurer 2010. Vitamin D metabolism. Dermatol Ther 23 (1): 2-12.

Lehtonen-Veromaa, M., T. Möttönen, A. Leino, O. J. Heinonen, E. Rautava and J. Viikari 2008. Prospective study on food fortification with vitamin D among adolescent females in Finland: minor effects. Br J Nutr 100 (2): 418-23.

Levine, B. S., F. R. Singer, G. F. Bryce, J. P. Mallon, O. N. Miller and J. W. Coburn 1985. Pharmacokinetics and biologic effects of calcitriol in normal humans. J Lab Clin Med 105 (2): 239-46.

Li-Ng, M., J. F. Aloia, S. Pollack, B. A. Cunha, M. Mikhail, J. Yeh and N. Berbari 2009. A randomized controlled trial of vitamin D3

supplementation for the prevention of symptomatic upper respiratory tract infections. Epidemiol Infect 137 (10): 1396-404.

Lips, P., R. Bouillon, N. M. van Schoor, D. Vanderschueren, S. Verschueren, N. Kuchuk, K. Milisen and S. Boonen 2010. Reducing fracture risk with calcium and vitamin D. Clin Endocrinol (Oxf) 73 (3): 277-85.

Lips, P. and N. M. van Schoor 2011. The effect of vitamin D on bone and osteoporosis. Best Pract Res Clin Endocrinol Metab 25 (4): 585-91.

Liu, P. T., S. Stenger, H. Li, L. Wenzel, B. H. Tan, S. R. Krutzik, M. T. Ochoa, J. Schauber, K. Wu, C. Meinken, D. L. Kamen, M. Wagner, R. Bals, A. Steinmeyer, U. Zugel, R. L. Gallo, D. Eisenberg, M. Hewison, B. W. Hollis, J. S. Adams, B. R. Bloom and R. L. Modlin 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311 (5768): 1770-3.

Lou, Y. R., I. Laaksi, H. Syvälä, M. Bläuer, T. L. Tammela, T. Ylikomi and P. Tuohimaa 2004. 25-hydroxyvitamin D3 is an active hormone in human primary prostatic stromal cells. Faseb J 18 (2): 332-4.

Lu, Z., T. C. Chen, A. Zhang, K. S. Persons, N. Kohn, R. Berkowitz, S. Martinello and M. F. Holick 2007. An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? J Steroid Biochem Mol Biol 103 (3-5): 642-4.

MacLaughlin, J. and M. F. Holick 1985. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 76 (4): 1536-8.

MacLaughlin, J. A., R. R. Anderson and M. F. Holick 1982. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science 216 (4549): 1001-3.

Margolis, R. N. and S. Christakos 2010. The nuclear receptor superfamily of steroid hormones and vitamin D gene regulation. An update. Ann N Y Acad Sci 1192 (1): 208-14.

Matsuoka, L. Y., L. Ide, J. Wortsman, J. A. MacLaughlin and M. F. Holick 1987. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab 64 (6): 1165-8.

Matsuoka, L. Y., J. Wortsman, M. J. Dannenberg, B. W. Hollis, Z. Lu and M. F. Holick 1992. Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3. J Clin Endocrinol Metab 75 (4): 1099-103.

Mattila, V. M., M. Niva, M. Kiuru and H. Pihlajamäki 2007. Risk factors for bone stress injuries: a follow-up study of 102,515 person-years. Med Sci Sports Exerc 39 (7): 1061-6.

Matusiak, D., G. Murillo, R. E. Carroll, R. G. Mehta and R. V. Benya 2005. Expression of vitamin D receptor and 25-hydroxyvitamin D3-1{alpha}-hydroxylase in normal and malignant human colon. Cancer Epidemiol Biomarkers Prev 14 (10): 2370-6.

McCarthy, K., C. Laban, S. A. Bustin, W. Ogunkolade, S. Khalaf, R. Carpenter and P. J. Jenkins 2009. Expression of 25-hydroxyvitamin D-1-alpha-

hydroxylase, and vitamin D receptor mRNA in normal and malignant breast tissue. Anticancer Res 29 (1): 155-7.

McClung, J. P. and J. P. Karl 2010. Vitamin D and stress fracture: the contribution of vitamin D receptor gene polymorphisms. Nutr Rev 68 (6): 365-9.

McCollum, E. V., N. Simmonds, M. Kinney, P. G. Shipley and E. A. Park 1995. Studies on experimental rickets. XVII. The effects of diets deficient in calcium and in fat-soluble A in modifying the histological structure of the bones. 1921. Am J Epidemiol 141 (4): 280-96; discussion 279.

McKenna, M. J. and R. Freaney 1998. Bone density and vitamin D intoxication. Ann Intern Med 128 (6): 507-8.

Mohr, S. B. 2009. A brief history of vitamin d and cancer prevention. Ann Epidemiol 19 (2): 79-83.

Myburgh, K. H., J. Hutchins, A. B. Fataar, S. F. Hough and T. D. Noakes 1990. Low bone density is an etiologic factor for stress fractures in athletes. Ann Intern Med 113 (10): 754-9.

Niva, M. H., V. M. Mattila, M. J. Kiuru and H. K. Pihlajamäki 2009. Bone stress injuries are common in female military trainees: a preliminary study. Clin Orthop Relat Res 467 (11): 2962-9.

Norman, A. W. 1998. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. Am J Clin Nutr 67 (6): 1108-10.

Norman, A. W. 2008. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 88 (2): 491S-499S.

Oilinki, T., T. Otonkoski, J. Ilonen, M. Knip and P. J. Miettinen 2012. Prevalence and characteristics of diabetes among Somali children and adolescents living in Helsinki, Finland. Pediatr Diabetes 13 (2): 176-80.

Pierrot-Deseilligny, C. and J. C. Souberbielle 2010. Is hypovitaminosis D one of the environmental risk factors for multiple sclerosis? Brain 133 (Pt 7): 1869-88.

Pietinen, P., S. Männisto, L. M. Valsta and S. Sarlio-Lähteenkorva 2010. Nutrition policy in Finland. Public Health Nutr 13 (6A): 901-6.

Pihlajamäki, H. K., J. P. Ruohola, M. J. Kiuru and T. I. Visuri 2006. Displaced femoral neck fatigue fractures in military recruits. J Bone Joint Surg Am 88 (9): 1989-97.

Pouilles, J. M., J. Bernard, F. Tremollieres, J. P. Louvet and C. Ribot 1989. Femoral bone density in young male adults with stress fractures. Bone 10 (2): 105-8.

Priemel, M., C. von Domarus, T. O. Klatte, S. Kessler, J. Schlie, S. Meier, N. Proksch, F. Pastor, C. Netter, T. Streichert, K. Puschel and M. Amling 2010. Bone mineralization defects and vitamin D deficiency:

histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. J Bone Miner Res 25 (2): 305-12.

Rachez, C. and L. P. Freedman 2000. Mechanisms of gene regulation by vitamin D(3) receptor: a network of coactivator interactions. Gene 246 (1-2): 9-21.

Renzaho, A. M., J. A. Halliday and C. Nowson 2011. Vitamin D, obesity, and obesity-related chronic disease among ethnic minorities: a systematic review. Nutrition 27 (9): 868-79.

Riggs, B. L. 1997. Vitamin D-receptor genotypes and bone density. N Engl J Med 337 (2): 125-6.

Ross, A. C., J. E. Manson, S. A. Abrams, J. F. Aloia, P. M. Brannon, S. K. Clinton, R. A. Durazo-Arvizu, J. C. Gallagher, R. L. Gallo, G. Jones, C. S. Kovacs, S. T. Mayne, C. J. Rosen and S. A. Shapses 2011. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J Clin Endocrinol Metab

Schleithoff, S. S., A. Zittermann, G. Tenderich, H. K. Berthold, P. Stehle and R. Koerfer 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 (4): 754-9.

Shaffer, R. A., S. K. Brodine, S. A. Almeida, K. M. Williams and S. Ronaghy 1999. Use of simple measures of physical activity to predict stress fractures in young men undergoing a rigorous physical training program. Am J Epidemiol 149 (3): 236-42.

Simkin, A., I. Leichter, M. Giladi, M. Stein and C. Milgrom 1989. Combined effect of foot arch structure and an orthotic device on stress fractures. Foot Ankle 10 (1): 25-9.

Smith, S. M., K. K. Gardner, J. Locke and S. R. Zwart 2009. Vitamin D supplementation during Antarctic winter. Am J Clin Nutr 89 (4): 1092-8.

Stamp, T. C., J. G. Haddad and C. A. Twigg 1977. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. Lancet 1 (8026): 1341-3.

Steenbock, H. 1924. The Induction of Growth Promoting and Calcifying Properties in a Ration by Exposure to Light. Science 60 (1549): 224-225.

Szulc, P., F. Munoz, F. Marchand, M. C. Chapuy and P. D. Delmas 2003. Role of vitamin D and parathyroid hormone in the regulation of bone turnover and bone mass in men: the MINOS study. Calcif Tissue Int 73 (6): 520-30.

Thacher, T. D., P. R. Fischer, M. O. Obadofin, M. A. Levine, R. J. Singh and J. M. Pettifor 2010. Comparison of metabolism of vitamins D(2) and D(3) in children with nutritional rickets. J Bone Miner Res 25 (9): 1988-1995.

Trang, H. M., D. E. Cole, L. A. Rubin, A. Pierratos, S. Siu and R. Vieth 1998. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. Am J Clin Nutr 68 (4): 854-8.

Trivedi, D. P., R. Doll and K. T. Khaw 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 (7387): 469.

Utiger, R. D. 1998. The need for more vitamin D. N Engl J Med 338 (12): 828-9.

Wang, T. T., F. P. Nestel, V. Bourdeau, Y. Nagai, Q. Wang, J. Liao, L. Tavera-Mendoza, R. Lin, J. W. Hanrahan, S. Mader and J. H. White 2004. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173 (5): 2909-12.

Webb, A. R., L. Kline and M. F. Holick 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 (2): 373-8.

Vieth, R. 1999. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 69 (5): 842-56.

Viljakainen, H. T., M. Väisänen, V. Kemi, T. Rikkonen, H. Kröger, E. Laitinen, H. Rita and C. Lamberg-Allardt 2008. Wintertime Vitamin D Supplementation Inhibits Seasonal Variation of Calcitropic Hormones and Maintains Bone Turnover in Healthy Men. J Bone Miner Res

Vlaminckx, B. J., W. van Pelt, L. M. Schouls, A. van Silfhout, E. M. Mascini, C. P. Elzenaar, T. Fernandes, A. Bosman and J. F. Schellekens 2005. Long-term surveillance of invasive group A streptococcal disease in The Netherlands, 1994-2003. Clin Microbiol Infect 11 (3): 226-31.

Wolpowitz, D. and B. A. Gilchrest 2006. The vitamin D questions: how much do you need and how should you get it? J Am Acad Dermatol 54 (2): 301-17.

von Essen, M. R., M. Kongsbak, P. Schjerling, K. Olgaard, N. Odum and C. Geisler 2010. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nat Immunol 11 (4): 344-9.

Wortsman, J., L. Y. Matsuoka, T. C. Chen, Z. Lu and M. F. Holick 2000. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 72 (3): 690-3.

Välimäki, V. V., H. Alfthan, E. Lehmuskallio, E. Loyttyniemi, T. Sahi, U. H. Stenman, H. Suominen and M. J. Välimäki 2004. Vitamin D status as a determinant of peak bone mass in young Finnish men. J Clin Endocrinol Metab 89 (1): 76-80.

Välimäki, V. V., H. Alfthan, E. Lehmuskallio, E. Löyttyniemi, T. Sahi, H. Suominen and M. J. Välimäki 2005. Risk factors for clinical stress fractures in male military recruits: a prospective cohort study. Bone 37 (2): 267-73.

Välimäki, V. V., E. Löyttyniemi and M. J. Välimäki 2007. Vitamin D fortification of milk products does not resolve hypovitaminosis D in young Finnish men. Eur J Clin Nutr 61 (4): 493-7.

Ylikomi, T., I. Laaksi, Y. R. Lou, P. Martikainen, S. Miettinen, P. Pennanen, S. Purmonen, H. Syvälä, A. Vienonen and P. Tuohimaa 2002. Antiproliferative action of vitamin D. Vitam Horm 64 357-406.

Zadshir, A., N. Tareen, D. Pan, K. Norris and D. Martins 2005. The prevalence of hypovitaminosis D among US adults: data from the NHANES III. Ethn Dis 15 (4 Suppl 5): S5-97-101.

Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms. Nature 415 (6870): 389-95.

Zasloff, M. 2006. Fighting infections with vitamin D. Nat Med 12 (4): 388-90.

Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men[1-3]

Ilkka T Laaksi, Juha-Petri S Ruohola, Timo J Ylikomi, Anssi P Auvinen, Riina I Haataja, Harri K Pihlajamäki, Pentti J Tuohimaa

[1] From the Department of Cell Biology, Medical School, University of Tampere, Finland (TJY, ITL), the Department of clinical chemistry, Tampere University Hospital, Finland (TJY, PJT), Garrison Hospital, Pori Brigade, Finnish Defence Forces, Säkylä, Finland (J-PSR), the Department of Epidemiology, Tampere School of Public Health, University of Tampere (AA), the Department of Biometrics, Tampere School of Public Health, University of Tampere Finland (RIH), the Department of Medical Services, Defence Staff, Finnish Defence Forces, Helsinki, Finland (HKP), the Institute of Military Medicine, Central Military Hospital, Helsinki, Finland (HKP) and the Department of anatomy, Medical School, University of Tampere, Finland (PJT)

[2] Supported by the grants from Tampere University Hospital, Finland, and the Academy of Finland.

[3] Address correspondence to Ilkka Laaksi, Department of Cell Biology, Medical School, 33014 University of Tampere Finland; Tel.: +358-3-35516661; Fax: +358-3-35516170; email: ilkka.laaksi@uta.fi

ABSTRACT

Background: Vitamin D insufficiency is common in northern countries during wintertime. In Finland, after the recommendation by the Ministry of Social Affairs and Health, vitamin D has been added to liquid milk products and margarines from February 2003.

Objective: We determined the effects of national policy on vitamin D fortification on vitamin D status among young Finnish men.

Design: A before and after intervention comparison with study population of 196 young Finnish men (18-28 y) was carried out. Serum 25-hydroxyvitamin D_3 (25-OHD_3) concentrations were determined with the OCTEIA® enzymeimmunoassay by IDS (Immunodiagnostic Systems Limited) in January 2003 (n=96) and in January 2004 (n=100), nearly 1 year after national vitamin D fortification had started.

Results: The mean serum 25-OHD_3 concentrations during the wintertime increased by 50% after implementation of the vitamin D fortification of dairy products. Correspondingly, the prevalence of vitamin D insufficiency (serum 25-OHD_3 <40 nmol/L) was decreased by 50% from 78 % in January 2003 to 35 % in January 2004.

Conclusions: Our results demonstrate that national vitamin D fortification substantially improved the vitamin D status of young Finnish men. Still, a third remained vitamin D insufficient.

KEYWORDS Vitamin D insufficiency, milk, vitamin D, vitamin D fortification, wintertime, 25-hydroxyvitamin D_3, 25-OHD_3

INTRODUCTION

Vitamin D regulates calcium and phosphate homeostasis by increasing their absorption from intestine and enhancing their renal reabsorption (Holick, 2003). This homeostasis is important for bone mineralization since in the case of inadequate calcium in the diet vitamin D causes osteoclasts to mature and dissolve calcium from bone. A low serum concentration of vitamin D leads to a lower serum calcium concentration and thereafter to an increased PTH secretion i.e. secondary hyperparathyroidism which follows that bone turnover increases (Lips, 2001). Rickets in childhood, mild osteomalacia, osteoporosis and an increased risk of hip and other fractures in adults are all known consequences of vitamin D deficiency (Compston, 1998; Utiger, 1998).

Vitamin D is also a hormone. Its genomic mechanism of action is mediated by vitamin D receptor which belongs to the family of steroid/thyroid hormone nuclear receptors. It has antiproliferative, differentiative and apoptotic effects on prostate cancer cells in vitro. (Ylikomi, Laaksi *et al.*, 2002) It has effects on cancer invasion and angiogenesis (Mantell, Owens *et al.*, 2000; Schwartz, Wang *et al.*, 1997; Ylikomi, Laaksi *et al.*, 2002). In epidemiological studies, vitamin D has been negatively associated to breast (Grant, 2002) and colon cancers (Garland, Comstock *et al.*, 1989). Vitamin D deficiency might also be associated to type I diabetes, hypertension, multiple sclerosis and some other cancers (Holick, 2004).

The main source of vitamin D is previtamin D_3 photolysed from 7-dehydrocholesterol in the skin by the UVB (290-315 nm) of sunlight exposure. This previtamin D_3 is then transformed to vitamin D_3 that is hydroxylated in liver to form 25-hydroxyvitamin D_3 (25-OHD_3). Lastly, 25-OHD_3 undergoes 1α-hydroxylation mainly in the kidney to form 1α, 25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$). (Holick, Uskokovic *et al.*, 1980)

Serum 25-OHD_3 is the best marker for the vitamin D status since both high serum concentrations in the summer and low concentrations in the winter have been observed in northern latitudes, reflecting the amount of exposure to the sun but it is thought to be a precursor for the most active vitamin D metabolite 1,25$(OH)_2D_3$ (Chapuy, Preziosi *et al.*, 1997). We have recently found evidence that 25-OHD_3 itself acts as an active hormone (Lou, Laaksi *et al.*, 2004).

Vitamin D sufficiency can be detected from the increase in serum parathyroid hormone (iPTH) secretion that begins to take place when serum 25-OHD_3 concentration falls below 78-90 nmol/l (Chapuy, Preziosi *et al.*, 1997; Harkness and Cromer, 2004; Krall, Sahyoun *et al.*, 1989). In Finland serum 25-OHD_3 concentration of 40 nmol/L is the limit that is clinically used for indicating vitamin D insufficiency.

In Europe, vitamin D insufficiency is common in children during wintertime. In elderly people it might last throughout the year and in institutionalized people serum 25-OHD_3 concentrations are even lower (Jacques, Felson *et al.*, 1997; McKenna, 1992; Zittermann, 2003). Furthermore, vitamin D insufficiency can be regarded as epidemic among adults without sufficient sunlight exposure (Holick, 2003).

In Finland, vitamin D insufficiency is very common among young men, young girls and healthy adults in wintertime (Lamberg-Allardt, Outila *et al.*, 2001; Valimaki, Alfthan *et al.*, 2004). Thus, from February 2003, after the recommendation by the Ministry of Social Affairs and Health, vitamin D has been added to liquid milk products (0.5 µg/dL), as well as margarines and butter (10 µg/100 g) in Finland.

Our aim was to study the effects of vitamin D fortification on the vitamin D status in young Finnish men.

SUBJECTS AND METHODS

Subjects

The study population was comprised of 196 young Finnish men (18-28 y) undergoing military training in the Finnish Defence Forces as conscripts. They had passed their arrival medical examination as healthy. In January 2003 a representative sample of subjects (n=96) was recruited at health examination covering all the conscripts (n=690). Similarly, a representative sample of subjects (n=100) was recruited at health examination covering all the conscripts (n=1158) in January 2004, nearly 1 year after national vitamin D fortification had started. The men were invited for the examination a unit at a time and each subject was asked to participate until the target number had been reached. Use of vitamin D supplementation and staying in sunny Southern countries during three previous months were used as exclusion criteria. The research was approved by the Ethical Committee of Tampere University Hospital, Finland. All the volunteers gave a written informed consent.

Methods

Serum 25-OHD_3 concentrations were determined with Octeia® enzyme immunoassay by IDS (Immunodiagnostic Systems Limited) in January 2003 (n=96) and in January 2004 (n=100). Serum 25-OHD_3 concentrations below 40 nmol/l were regarded as indicating vitamin D insufficiency.

RESULTS

The mean daily intake of milk and margarine among military conscripts were 8 dL and 30 g, respectively. After fortification the young men were ingesting on average 7 µg i.e. 280 IU of

vitamin D (8 dL x 0.5 µg/dL + 30 g x 10 µg/100g), daily. The mean serum 25-OHD_3 concentrations during the wintertime increased by 50% after implementation of the vitamin D fortification of dairy products **(Table)**. Correspondingly, the prevalence of vitamin D insufficiency (serum 25-OHD_3 <40 nmol/L) was decreased by 50 % from 78 % in January 2003 to 35 % in January 2004.

The prevalence of vitamin D deficiency (serum 25-OHD_3 <25 nmol/L) decreased from 19 % in January 2003 to 5 % in January 2004.

After fortification, five subjects (5 %) had serum 25-OHD_3 concentration higher than 100 nmol/L (101-111 nmol/L).

Table.

Serum 25-OHD_3 levels in young Finnish men

	January 2003		January 2004		
					Difference in means (95% CI)*
Number (total)	96		100		
Mean, nmol/l (±SD)	33.5	(9.2)	50.2	(20.3)	16.69 (12.27, 21.11)
25-OHD_3 concentration					Prevalence Ratio (95% CI)**
< 40.0 nmol/l	75	(78.1)	35	(35.0)	0.45 (0.34, 0.60)
40.0-59.9 nmol/l	20	(20.8)	41	(41.0)	1.97 (1.25, 3.11)
60.0-99.9 nmol/l	1	(1.0)	19	(19.0)	19.00 (2.59, 139.18)
≥ 100 nmol/l	-	(0.0)	5	(5.0)	∞

*Change from Jan 2003 to Jan 2004
**Jan 2004 relative to Jan 2003 within each category 25-OHD_3

DISCUSSION

Our results demonstrate that national vitamin D fortification substantially improved the vitamin D status of young Finnish men. The mean serum 25-OHD_3 concentrations were 50 % higher than those measured before the vitamin D fortification. The fortification significantly diminished but did not remove vitamin D insufficiency in young men during wintertime, since a third remained vitamin D insufficient. In 2003, 18 of 96 study subjects had their serum 25-OHD_3 concentrations lower than 25 nmol/L indicating vitamin D deficiency. However, in 2004 nearly 1 year after vitamin D fortification only 5 of 100 subjects had vitamin D deficiency.

After fortification, five subjects (5 %) had serum 25-OHD_3 concentration higher than 100 nmol (101-111 nmol/L). However, no clinical signs indicating toxicity were seen, though information on symptoms was not systematically collected. Still, when vitamin D is added in dairy products, there is always a possibility of overdosing in some individuals.

Vitamin D fortification covers liquid milk products, (0,5 µg D_3/ 100 ml) margarines and butter (10 µg/100g). Practically all manufacturers have complied with the recommendation in Finland.

Based on average daily intake of milk and margarine in the Finnish Defence Forces these young men were ingesting 7 µg i.e. 280 IU of vitamin D daily after fortification, corresponding to the target level of the Ministry of Social Affairs and Health in Finland. We did not have information on vitamin D intake at individual level, as no dietary information was collected by e.g. questionnaires.

All Finnish men become liable for a 6, 9, or 12-month-long military service at the age of 18 years. Approximately 90 % of Finnish men serve in the military. Hence, the military conscripts comprise the vast majority of all young men at the same age in Finland. Yet, diet during military service may differ from the rest of the population and those with major long-standing illness are exempted from military service.

Children and elderly people consume milk products less than military conscripts in the study and are high risk groups for vitamin D insufficiency. They will probably benefit most from the vitamin D fortification as a national policy.

We also need to consider people not ingesting milk products. Interestingly, a highly significant rise in serum 25-OHD_3 concentrations (from 37 to 94 nmol/L) was shown in subjects ingesting a daily glass of orange juice fortified with 1000 IU vitamin D_3 for 12 weeks. In the same study a significant 25 % decrease in PTH concentrations associated with a 20 % decrease in the concentration of urine N-telopeptide, a marker for bone turnover were show. Both milk and juice proved suitable vehicle foods in which the vitamin D is placed. (Tangpricha, Koutkia *et al.*, 2003)

Due to its longer distance through the atmosphere the amount of UVB in sunshine is insufficient for vitamin D production during wintertime at northern latitudes. In Edmonton (52º N) the ineffective winter period lasts from October to March (Webb, Kline *et al.*, 1988). In Finland, location between 60º and 70º N, that period is even longer.

We measured serum 25-OHD_3 concentrations in January because the midwinter values better reflect the long duration of vitamin D insufficiency during wintertime. However, it is likely that the lowest serum 25-OHD_3 concentrations are found at the end of wintertime suggesting vitamin D insufficiency still to be found in substantially more than 1/3 of young men in Finland. The long-term effects on health, however, remain to be demonstrated.

References

Chapuy, M. C., P. Preziosi, et al. (1997). "Prevalence of vitamin D insufficiency in an adult normal population." Osteoporos Int **7**(5): 439-43.

Compston, J. E. (1998). "Vitamin D deficiency: time for action. Evidence supports routine supplementation for elderly people and others at risk." Bmj **317**(7171): 1466-7.

Garland, C. F., G. W. Comstock, et al. (1989). "Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study." Lancet **2**(8673): 1176-8.

Grant, W. B. (2002). "An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates." Cancer **94**(1): 272-81.

Harkness, L. and B. Cromer (2004). "Low levels of 25-hydroxy vitamin D are associated with elevated parathyroid hormone in healthy adolescent females." Osteoporos Int.

Holick, M. F. (2003). "Evolution and function of vitamin D." Recent Results Cancer Res **164**: 3-28.

Holick, M. F. (2003). "Vitamin D: A millenium perspective." J Cell Biochem **88**(2): 296-307.

Holick, M. F. (2004). "Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis." Am J Clin Nutr **79**(3): 362-71.

Holick, M. F., M. Uskokovic, et al. (1980). "The photoproduction of 1 alpha,25-dihydroxyvitamin D3 in skin: an approach to the therapy of vitamin-D-resistant syndromes." N Engl J Med **303**(7): 349-54.

Jacques, P. F., D. T. Felson, et al. (1997). "Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample." Am J Clin Nutr **66**(4): 929-36.

Krall, E. A., N. Sahyoun, et al. (1989). "Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women." N Engl J Med **321**(26): 1777-83.

Lamberg-Allardt, C. J., T. A. Outila, et al. (2001). "Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe?" J Bone Miner Res **16**(11): 2066-73.

Lips, P. (2001). "Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications." Endocr Rev **22**(4): 477-501.

Lou, Y. R., I. Laaksi, et al. (2004). "25-hydroxyvitamin D3 is an active hormone in human primary prostatic stromal cells." Faseb J **18**(2): 332-4.

Mantell, D. J., P. E. Owens, et al. (2000). "1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo." Circ Res **87**(3): 214-20.

McKenna, M. J. (1992). "Differences in vitamin D status between countries in young adults and the elderly." Am J Med **93**(1): 69-77.

Schwartz, G. G., M. H. Wang, et al. (1997). "1 alpha,25-Dihydroxyvitamin D (calcitriol) inhibits the invasiveness of human prostate cancer cells." Cancer Epidemiol Biomarkers Prev **6**(9): 727-32.

Tangpricha, V., P. Koutkia, et al. (2003). "Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health." Am J Clin Nutr **77**(6): 1478-83.

Utiger, R. D. (1998). "The need for more vitamin D." N Engl J Med **338**(12): 828-9.

Valimaki, V. V., H. Alfthan, et al. (2004). "Vitamin D status as a determinant of peak bone mass in young Finnish men." J Clin Endocrinol Metab **89**(1): 76-80.

Webb, A. R., L. Kline, et al. (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**(2): 373-8.

Ylikomi, T., I. Laaksi, et al. (2002). "Antiproliferative action of vitamin D." Vitam Horm **64**: 357-406.

Zittermann, A. (2003). "Vitamin D in preventive medicine: are we ignoring the evidence?" Br J Nutr **89**(5): 552-72.

JOURNAL OF BONE AND MINERAL RESEARCH
Volume 21, Number 9, 2006
Published online on June 19, 2006; doi: 10.1359/JBMR.060607

Association Between Serum 25(OH)D Concentrations and Bone Stress Fractures in Finnish Young Men

Juha-Petri Ruohola,[1,2] Ilkka Laaksi,[2,3] Timo Ylikomi,[3,4] Riina Haataja,[5,6] Ville M Mattila,[1] Timo Sahi,[7] Pentti Tuohimaa,[4,8] and Harri Pihlajamäki[1]

ABSTRACT: Low vitamin D level may predict rickets, osteomalacia, or osteoporosis. We examined serum 25(OH)D concentration as a predisposing factor for bone stress fracture in 756 military recruits. The average serum 25(OH)D concentration was significantly lower in the group with fracture, suggesting a relationship between vitamin D and fatigue bone stress fracture.

Introduction: Low vitamin D level may predict rickets, osteomalacia, or osteoporosis. Fatigue bone stress fracture is one of the most frequently seen types of overuse injuries in athletes and military recruits. An association was recently shown between vitamin D and BMC. A correlation has also been found between low femoral BMD and stress fractures. We measured serum 25(OH)D concentration in a population sample of military recruits to determine if vitamin D is a predisposing factor for fatigue bone stress fracture.
Materials and Methods: We prospectively followed 800 randomly selected, healthy Finnish military recruits with a mean age of 19 years for developing stress fractures in homogenous circumstances. Blood for serum 25(OH)D concentration was drawn at entry into military service, and the weight, height, body mass index (BMI), muscle strength, and 12-minute running were measured for all subjects. Serum 25(OH)D concentrations were measured with enzyme immunoassay. At end of the 90-day follow-up, 756 subjects completed the study. Subjects without fracture constituted controls.
Results: Twenty-two recruits with stress fracture were identified (2.9%), the incidence being 11.6 (95% CI: 6.8–16.5) per 100 person-years. In the final multivariate analysis, the significant risk factor for stress fracture in conscripts was a below median serum 25(OH)D level (75.8 nM), OR being 3.6 (95% CI: 1.2–11.1). No significant associations between BMI ($p = 0.255$), age ($p = 0.216$), or smoking ($p = 0.851$) and bone stress fracture were found in this study population.
Conclusions: A lower level of serum 25(OH)D concentration may be a generally predisposing element for bone stress fractures. Considering the obvious need of additional vitamin D in prevention of stress fractures, the effects of vitamin D fortification of foods and supplementation will be subjects of interest for future research.
J Bone Miner Res 2006;21:1483–1488. Published online on June 19, 2006; doi: 10.1359/JBMR.060607

Key words: vitamin D, 25(OH)D, bone, stress fracture, young men

INTRODUCTION

BONE STRESS FRACTURE is a common overuse injury in athletes and military recruits.[1–3] Two main types of bone stress fractures are recognized: fractures induced by cyclic loading of normal bones with abnormal forces are fatigue fractures and those induced by normal forces in abnormal bone are insufficiency fractures.[4–6] Normally, bone responds to strain by remodeling. To excessive strain, the bone reacts with accelerated remodeling, accumulation of microfractures, and bone fatigue that may result in stress fracture.[7] Some high-risk stress fractures, such as fractures in the femoral neck, may progress into severe complications such as delayed union, nonunion, malunion, avascular necrosis, and osteoarthritis.[8,9] Stress injuries of the bone are associated with intensive, or recently intensified, physical activity among, for example, athletes and military trainees.[4] The incidence of stress fractures has been shown to remain <3.7% in the general athletic population, representing 10% of all sports injuries seen in clinics.[1,3,10] However, in some groups of athletes, such as runners, these numbers can be relatively higher.[1,8,11,12] The current published incidences in military recruits vary between 0.9% and 12.3%,

The authors state that they have no conflicts of interest.

[1]Research Institute of Military Medicine, Helsinki, Finland; [2]These authors contributed equally to this paper; [3]Department of Cell Biology, Medical School, University of Tampere, Tampere, Finland; [4]Department of Clinical Chemistry, Tampere University Hospital, Tampere, Finland; [5]Tampere School of Public Health, University of Tampere, Tampere, Finland; [6]Research Unit, Tampere University Hospital, Tampere, Finland; [7]Department of Health, Defence Staff, Helsinki, Finland; [8]Department of Anatomy, Medical School, University of Tampere, Tampere, Finland.

but incidences as high as 31% have been reported.[13–18] In military trainees and athletes, nearly all stress fractures are located in the lower extremities or the pelvis.[1,3,11,12,16,19,20]

Of the risk factors for stress fractures, female gender, age, body composition, bone characteristics, low BMD and bone strength, low aerobic fitness, low past physical activity level, smoking, and excess running have been identified in an epidemiologic review.[12,21] Pouilles et al.[22] found a correlation between low femoral BMD and stress fractures in military recruits. Bone scintigraphy or MRI of the lower extremities or pelvis often reveal multiple simultaneous bone stress injuries.[19,20,23,24]

Vitamin D is one nutrient thought to contribute to bone health. 1,25-dihydroxyvitamin D, the physiologically most active vitamin D metabolite, acts through the vitamin D receptor directly to increase intestinal calcium absorption and to enhance renal calcium reabsorption.[25] It is also known to decrease PTH secretion (iPTH) and enhance the differentiation of both osteoblast and osteoclast precursors.[25–27] Calcium and phosphate homeostasis has a major effect on bone mineralization and peak bone mass, because if dietary calcium is inadequate vitamin D causes osteoclasts to mature and resorb calcium from the bone.[28–30]

Vitamin D deficiency is known to lead to rickets or osteomalacia, and vitamin D insufficiency is involved in the development of osteoporosis. As shown in elderly people, the low 25(OH)D levels reflecting the vitamin D status are associated with reduced BMD at the proximal femur.[31,32] Interestingly, this association has been verified for forearm BMD in female adolescents as well.[33,34] Recently, in a study of 220 young Finnish men, an association between vitamin D and BMC was found as men with above median levels for serum 25(OH)D had a higher BMC at the trochanter and total hip than men with lower levels.[28] An increase in BMD has been described in studies involving vitamin D supplementation.[35,36] Moreover, a rise in the mean 25(OH)D level from 53 to 74 nM through vitamin D supplementation has been shown to reduce fracture risk at hip, forearm, and spine in people ≥65 years of age.[37] Furthermore, serum 25(OH)D levels <50 nM have been noted more often in patients with hip fractures than in control subjects of the same age.[34,38] Previous studies showed that, when combined with calcium, vitamin D is capable of preventing nonvertebral fractures in healthy subjects.[39,40] Some earlier studies have observed a possible relationship of calcium intake with stress fracture development, but the actual evidence of such claim is still lacking.[41,42] In comparison, Välimäki et al.[28] found no association between serum iPTH and calcium intake. Vitamin D sufficiency is shown by an increase in iPTH secretion, initiated when serum 25(OH)D concentration falls <78–90 nM.[43–45] The increased iPTH secretion has been shown to augment bone turnover and contribute to bone fragility.[31,33]

Following the hypothesis that a factor might exist that generally predisposes to bone stress fracture, we assessed the effect of serum 25(OH)D, a precursor for active vitamin D metabolite 1,25-dihydroxyvitamin D, concentration as a predisposing factor. The incidence and anatomic distribution of these fatigue bone stress injuries and their relationship with the age, weight, height, BMI, muscle strength, and result of running test were also examined.

MATERIALS AND METHODS

For this prospective study, 800 Finnish young men undergoing military training in the Finnish Defense Forces as recruits of the same infantry unit were randomly selected. They were receiving no medication, including vitamin D supplementation, and they passed their entry medical examination as healthy. The subjects represented the general recruit population of the Finnish Defense Forces, with no specific features. The conditions related to physical activity, nutrition, clothing, accommodation, and exposure to sunlight were homogenous during their military service. All Finnish men become liable for a mandatory 6-, 9-, or 12-month military service at the age of 18. Military service is voluntary for women. Annually, on average, 26,500 male recruits and 500 female recruits undergo military training. This study was approved by the Ethical Committee of Tampere University Hospital, Finland. Participation was voluntary, and a written informed consent was obtained from all participants.

Blood samples for the determination of the serum 25(OH)D concentration were drawn from these 800 recruits (range, 18–28 years; mean, 19.8 years) at entry into military service in the beginning of July. Because of failed samples, incomplete follow-up data, and interrupted military services, the total study population was comprised of 756 subjects. After coagulation at room temperature for 1 h, the samples were centrifuged at 2000*g* for 20 minutes at room temperature for serum separation. The serum samples were frozen and stored at –20°C for later analysis. Total serum 25(OH)D concentrations were measured with OCTEIA enzyme immunoassay by IDS (Immunodiagnostic Systems, Fountain Hills, AZ, USA). The IDS intra- and interassay CVs for the assay are <8% and <10%, with a precision range of 5.3–380 nM (CV < 10%). The cross-reactivities for $25(OH)D_3$ and $25(OH)D_2$ are 100% and 75%, respectively. The 25(OH)D enzyme immunoassay (EIA) correlates well with radioimmunoassay (RIA), $r^2 = 0.82$.

Information on the recruits' background variables, such as physical fitness, was collected. The available computer-based statistics included data on the age, sex, height, and weight of every recruit. The BMI of all recruits was calculated by dividing the body weight in kilograms by the square of body height in meters. Information about the aerobic fitness and muscle strength used in this study had been obtained during the first weeks of the service. Aerobic fitness was measured using a 12-minute running test (CV = 0.12). Muscle strength was assessed with five measures (distance of horizontal jump and number of sit-ups, push-ups, pull-ups, and back-lifts) using in each a range from 0 to 3 points. The CV was 0.18. Information about daily smoking was assessed from questionnaires, to which the response rate was low (38%).

For 90 days, from early July, we followed the subjects to identify possible stress injuries to bone, because the majority of stress fractures of our interest occur during the first 8-week basic training period equal to all military recruits in

Table 1. Characteristics of the Study Population by Stress Fracture Status

Risk factor	*With stress fracture (n = 22)*	*Without stress fracture (n = 734)*	*Significance (test)*
Median (range)			
Concentration of 25(OH)D (nM)	64.3 (40.1–159.0)	76.2 (25.2–259.0)	0.017 (M-W)
Number (frequency)			
25(OH)D (nM)			
Less than median	18 (81.8%)	362 (49.3%)	0.002 (P)
Greater than or equal to median (75.8 nM)	4 (18.2%)	372 (50.7%)	
Missing *N*	0	0	
Daily smoking			
Yes	7 (36.8)	93 (34.7)	0.85 (P)
No	12 (63.2)	175 (65.3)	
Missing	3	466	
Mean (range)			
Age (years)	20.0 (18.6–22.3)	19.8 (18.0–28.5)	0.27 (T)
Missing *N*	0	0	
BMI (kg/m^2)	24.0 (15.4–37.4)	23.2 (16.6–39.2)	0.41 (T)
Missing *N*	1	14	
Height (cm)	177 (168–184)	179 (161–203)	0.15 (T)
Missing *N*	1	14	
Weight (kg)	75.3 (47.2–121.1)	74.3 (50.3–139.4)	0.70 (T)
Missing *N*	1	13	
Muscle strength	7 (0–15)	9 (1–15)	0.025 (T)
Missing *N*	67	67	
12-minute run (m)	2480 (1650–3200)	2670 (1540–3580)	0.007 (T)
Missing *N*	0	49	

M-W, Mann-Whitney *U*-test; P, Pearson χ^2 test; T, Student's *t*-test.

Finland.[3] In the beginning of the study, all the recruits of the study group were informed about the study and instructed to seek medical attention in the event that symptoms suggestive of bone stress injuries occurred. All patients who by clinical examination with careful history-taking were suspected to have sustained a bone stress injury underwent plain radiographic imaging with accepted radiological assessment.[11,18] The gray cortex sign, endosteal callus, periosteal callus, and fracture line were accepted as the radiographic signs marking a stress injury to bone. In addition, cases with normal findings on plain radiography seeking medical advice for the second time caused by prolonged exercise-induced pain in the pelvic area or the lower extremities were referred to MRI examination to the main military hospital. Moreover, patients with stress fracture on MRI were included in the study. The remainder of the subjects under observance constituted controls for the stress fracture cases.

At stage 1 in the statistical analysis, the skew continuous serum 25(OH)D values were divided into two categories based on the median. The differences in serum 25(OH)D between the groups were tested by Pearson χ^2 test. The results were corroborated by Mann-Whitney's *U*-test using the original values. Student's *t*-test was used to test differences in age, BMI, height, weight, muscle strength, and 12-minute running between the groups. The limit for statistical significance was set equal to 0.05. Data analysis was performed using SPSS for Windows (version 11.0; SPSS, Chicago, IL, USA).

At stage 2 in statistical analysis, logistic regression was applied to study the association between stress fracture and the significant explanatory variables from stage 1. ORs were calculated with a 95% CI. Variables showing a statistical significance at stage 1 [dichotomized serum 25(OH)D concentration, muscle strength, and 12-minute running] were included in the same forward stepwise regression model. Data analysis was performed using SPSS for Windows (version 12.0; SPSS).

RESULTS

During the 3-month follow-up of this population of 756 recruits, 22 persons with stress fractures were identified. The median serum 25(OH)D level in the group of recruits with stress fracture was significantly lower than in the group of recruits without stress fracture ($n = 734$; Table 1). The median level for serum 25(OH)D was 75.8 nM (range, 25.2–259.0 nM) for all the subjects in this study. The subjects with serum 25(OH)D levels below the median had a statistically significantly greater risk for bone stress fracture than those above the median (Table 1).

The incidence of stress fractures in this study was 11.6 (95% CI, 6.8–16.5) per 100 person-years (2.9%). In the 22 patients, plain radiography revealed 30 stress fractures in total: 13 (43%) were located in the tibia, 10 (33%) in the metatarsal bones, 3 (10%) in the calcaneus, and 2 (7%) in the tarsal navicular bone. In the inferior ramus and the femur, one fracture was found in each. Thirteen bone stress injuries were located in the right lower limb and 17 in the left. A developing stress injury became symptomatic on average 39 days (range, 4–70 days) after starting military training.

In this study population, recruits with stress fractures had significantly poorer results in the 12-minute running test

Table 2. ORs of Stress Fracture From Forward Stepwise Multivariate Logistic Regression Models

Risk factor	*OR (95% CI)*
25(OH)D (nM)	
Less than median	3.6 (1.2–11.1)
Greater than or equal to median (75.8 nM)	1 (reference)
Cooper test/12-minute run (continuous)	0.999 (0.997–1.000)
Muscle strength (continuous)	Not significant

(mean, 2480 versus 2670 m; $p = 0.007$) and in muscle strength (mean, 7 versus 9; $p = 0.025$) than controls. No significant association between daily smoking ($p = 0.85$), BMI ($p = 0.41$), age ($p = 0.27$), height ($p = 0.15$), and weight ($p = 0.70$) and bone stress fracture was found (Table 1.).

In the multivariate regression model, which adjusted all significant variables from stage 1, the risk of stress fracture in conscripts with serum 25(OH)D levels below the median was 3.6 (95% CI: 1.2–11.1) times the risk of those with concentrations exceeding the median level. The results of muscle strength or 12-minute running tests were not significantly associated with stress fractures in the multivariate model (Table 2).

DISCUSSION

The principal finding of this prospective study was that the average serum 25(OH)D concentration reflecting vitamin D status was significantly lower in the recruits who sustained a bone stress fracture than in the group of controls. This correlation between the low levels of vitamin D and bone stress fractures has not yet been published. However, a low serum concentration of vitamin D leads to a lower serum calcium concentration, and this in turn will increase PTH secretion, leading to secondary hyperparathyroidism. It follows that the bone turnover accelerates.[29,30] High serum PTH levels were recently found to be a risk factor for stress fracture in male military recruits in Finland.[21] Together, the aforementioned finding and our results strongly support the likelihood that a relationship exists between inadequate vitamin D and bone stress fractures.

Furthermore, in our study, recruits with a serum 25(OH)D concentration <76 nM exhibited a significantly higher number of stress fractures than recruits with a higher concentration. Chapuy et al.[44] found a negative correlation between serum PTH and 25(OH)D levels, indicating that serum PTH held a stable plateau level while serum 25(OH)D levels exceeded 78 nM, but increased when 25(OH)D levels dropped below that. In the elderly population, serum 25(OH)D threshold levels as high as 100 nM have been found.[46] In addition, a change in serum 25(OH)D concentration from 50 to 86 nM significantly increased calcium absorption in the intestine.[47] Comparing to previous papers, our results suggest that secondary hyperparathyroidism and the accelerated bone turnover increasing the risk of bone stress fracture are avoidable if serum 25(OH)D levels exceed 78–100 nM throughout the year.[44,48,49]

In this study, poor physical fitness was not associated with stress fractures in the multivariate model, although muscle strength and 12-minute running were significant in the univariate analysis. Some previous papers have been in concordance with findings of this study.[12,18,21,23] However, according to the previous literature, the risk factors for stress fractures are a controversial issue. In a large series from Lappe et al.[18] ($n = 4139$) of female U.S. Army recruits, no association was found between the height, weight, and BMI of the recruits and the risk of bone stress fracture. Likewise, in the studies performed in the Israeli Defense Forces, no significant association was found between height, weight, or BMI and the stress fracture incidence.[23,50] Furthermore, Lappe et al., consistent with many other military studies, reported that older age may increase the risk of stress fracture.[12,14,18] Moreover, the major finding in the aforementioned study was that a calcaneal quantitative low ultrasound measurement was predictive of stress fracture. Their finding is contradictory to the results of this study and those of Välimäki et al.[21] from a recent Finnish study. However, that same Finnish study implies that tallness is a risk factor for stress fracture.[21] The prevalence and distribution of bone stress fractures in this study were quite as expected and equal to those documented in previous publications.[3,11–14,17,19]

Results from studies in the Israeli Defense Forces showed that patients with scintigraphically diagnosed high-grade stress fractures also displayed significantly lower serum levels of 25(OH)D compared with control groups.[15] In comparison, the absolute level values in this study were fully equal to those in Israel. Considering vitamin D level's role as a predisposing factor for stress injuries in view of the similar findings in two countries and the evidence that inadequate sunlight leads to vitamin D insufficiency even among young people,[28,51] we may suppose that there is a substantial risk for stress fractures of the bone in Finland. In Israel, the sun shines almost all year round, whereas in Northern Europe during wintertime, the amount of sunlight fails to provide sufficient vitamin D production.

Among the group of controls, we found that in only 27 (3.7%) cases was the serum 25(OH)D concentration <40 nM, which in Finland is the limit clinically used for indicating vitamin D insufficiency.[35] Furthermore, no vitamin D insufficiency was evident from the mean serum 25(OH)D values, either in the group of controls or in the group with bone stress fractures. However, we measured these serum 25(OH)D concentrations in July, when the vitamin D status was expected to be at its annual peak and to stay high until late autumn.

This study showed that a lower level of serum 25(OH)D concentration may be a generally predisposing element for bone stress fractures. Recently, after this study was performed, by recommendation of the Finnish Ministry of Social Affairs and Health, vitamin D has been added to commercially produced milk (0.5 μg/100 ml) and certain margarines (10 μg/100 g), with the aim of raising the consumption of vitamin D from an average of 4 to 7 μg/day per

person. In view of the findings of this study, highlighting an obvious need for additional vitamin D to prevent stress fractures, future research should continue to explore the possible effects of vitamin D fortification as part of the national public health policy.

ACKNOWLEDGMENTS

This study was supported by the grants from Scientific Committee of National Defence, Finland; Tampere University Hospital, Finland; and the Academy of Finland.

REFERENCES

1. Matheson GO, Clement DB, McKenzie DC, Taunton JE, Lloyd-Smith DR, MacIntyre JG 1987 Stress fractures in athletes: A study of 320 cases. Am J Sports Med **15:**46–58.
2. Sterling JC, Edelstein DW, Calvo RD, Webb R II 1992 Stress fractures in the athlete: Diagnosis and management. Sports Med **14:**336–346.
3. Jones BH, Harris JM, Vinh TN, Rubin C 1989 Exercise-induced stress fractures and stress reactions of bone: Epidemiology, etiology, and classification. Exerc Sport Sci Rev **17:**379–422.
4. Pentecost RL, Murray RA, Brindley HH 1964 Fatigue, insufficiency, and pathologic Fractures. JAMA **187:**1001–1004.
5. Daffner RH, Pavlov H 1992 Stress fractures: Current concepts. AJR Am J Roentgenol **159:**245–252.
6. Anderson MW, Greenspan A 1996 Stress fractures. Radiology **199:**1–12.
7. Li GP, Zhang SD, Chen G, Chen H, Wang AM 1985 Radiographic and histologic analyses of stress fracture in rabbit tibias. Am J Sports Med **13:**285–294.
8. Boden BP, Osbahr DC 2000 High-risk stress fractures: Evaluation and treatment. J Am Acad Orthop Surg **8:**344–353.
9. Visuri T, Vara A, Meurman KO 1988 Displaced stress fractures of the femoral neck in young male adults: A report of twelve operative cases. J Trauma **28:**1562–1569.
10. Goldberg B, Pecora C 1994 Stress fractures: A risk of increased training in freshman. Physician Sports Med **22:**68–78.
11. Kiuru MJ, Pihlajamäki HK, Ahovuo JA 2004 Bone stress injuries. Acta Radiol **45:**317–326.
12. Jones BH, Thacker SB, Gilchrist J, Kimsey CD Jr, Sosin DM 2002 Prevention of lower extremity stress fractures in athletes and soldiers: A systematic review. Epidemiol Rev **24:**228–247.
13. Macleod MA, Houston AS, Sanders L, Anagnostopoulos C 1999 Incidence of trauma related stress fractures and shin splints in male and female army recruits: Retrospective case study. BMJ **318:**29.
14. Brudvig TJ, Gudger TD, Obermeyer L 1983 Stress fractures in 295 trainees: A one-year study of incidence as related to age, sex, and race. Mil Med **148:**666–667.
15. Givon U, Friedman E, Reiner A, Vered I, Finestone A, Shemer J 2000 Stress fractures in the Israeli defense forces from 1995 to 1996. Clin Orthop **373:**227–232.
16. Milgrom C, Giladi M, Stein M, Kashtan H, Margulies JY, Chisin R, Steinberg R, Aharonson Z 1985 Stress fractures in military recruits: A prospective study showing an unusually high incidence. J Bone Joint Surg Br **67:**732–735.
17. Armstrong DW III, Rue JPH, Wilckens JH, Frassica FJ 2004 Stress fracture injury in young military men and women. Bone **35:**806–816.
18. Lappe J, Davies K, Recker R, Heaney R 2005 Quantitative ultrasound: Use in screening for susceptibility to stress fractures in female army recruits. J Bone Miner Res **20:**571–578.
19. Kiuru MJ, Pihlajamäki HK, Hietanen HJ, Ahovuo JA 2002 MR imaging, bone scintigraphy, and radiography in bone stress injuries of the pelvis and the lower extremity. Acta Radiol **43:**207–212.
20. Ha KI, Hahn SH, Chung MY, Yang BK, Yi SR 1991 A clinical study of stress fractures in sports activities. Orthopedics **14:**1089–1095.
21. Välimäki VV, Alfthan H, Lehmuskallio E, Löyttyniemi E, Sahi T, Suominen H, Välimäki MJ 2005 Risk factors for clinical stress fractures in male military recruits: A prospective cohort study. Bone **37:**267–273.
22. Pouilles JM, Bernard J, Tremollieres F, Louvet JP, Ribot C 1989 Femoral bone density in young male adults with stress fractures. Bone **10:**105–108.
23. Giladi M, Milgrom C, Simkin A, Danon Y 1991 Stress fractures: Identifiable risk factors. Am J Sports Med **19:**647–652.
24. Niva MH, Kiuru MJ, Haataja R, Pihlajamäki HK 2005 Fatigue injuries of the femur. J Bone Joint Surg Br **87:**1385–1390.
25. Utiger RD 1998 The need for more vitamin D. N Engl J Med **338:**828–829.
26. Riggs BL 1997 Vitamin D-receptor genotypes and bone density. N Engl J Med **337:**125–126.
27. Holick MF 2003 Vitamin D: A millenium perspective. J Cell Biochem **88:**296–307.
28. Välimäki VV, Alfthan H, Lehmuskallio E, Löyttyniemi E, Sahi T, Stenman UH, Suominen H, Välimäki MJ 2004 Vitamin D status as a determinant of peak bone mass in young Finnish men. J Clin Endocrinol Metab **89:**76–80.
29. Lips P 2001 Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. Endocr Rev **22:**477–501.
30. Compston JE 1998 Vitamin D deficiency: Time for action: Evidence supports routine supplementation for elderly people and others at risk. BMJ **317:**1466–1467.
31. Szulc P, Munoz F, Marchand F, Chapuy MC, Delmas PD 2003 Role of vitamin D and parathyroid hormone in the regulation of bone turnover and bone mass in men: The MINOS study. Calcif Tissue Int **73:**520–530.
32. Zittermann A 2003 Vitamin D in preventive medicine: Are we ignoring the evidence? Br J Nutr **89:**552–572.
33. Cheng S, Tylavsky F, Kroger H, Karkkainen M, Lyytikainen A, Koistinen A, Mahonen A, Alen M, Halleen J, Vaananen K, 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. Am J Clin Nutr **78:**485–492.
34. McKenna MJ 1992 Differences in vitamin D status between countries in young adults and the elderly. Am J Med **93:**69–77.
35. Vieth R 1999 Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr **69:**842–856.
36. Holick MF 2003 Evolution and function of vitamin D. Recent Results Cancer Res **164:**3–28.
37. Trivedi DP, Doll R, Khaw KT 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.
38. Jacques PF, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH, Rush D 1997 Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. Am J Clin Nutr **66:**929–936.
39. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ 1992 Vitamin D3 and calcium to prevent hip fractures in the elderly women. N Engl J Med **327:**1637–1642.
40. Lamberg-Allardt CJ, Outila TA, Karkkainen MU, Rita HJ, Valsta LM 2001 Vitamin D deficiency and bone health in healthy adults in Finland: Could this be a concern in other parts of Europe? J Bone Miner Res **16:**2066–2073.
41. Lips P, Netelenbos JC, van Doorn L, Hackeng WH, Lips CJ 1991 Stimulation and suppression of intact parathyroid hormone (PTH1-84) in normal subjects and hyperparathyroid patients. Clin Endocrinol (Oxf) **35:**35–40.
42. McKane WR, Khosla S, Egan KS, Robins SP, Burritt MF, Riggs BL 1996 Role of calcium intake in modulating age-related increases in parathyroid function and bone resorption. J Clin Endocrinol Metab **81:**1699–1703.

43. Harkness L, Cromer B 2005 Low levels of 25-hydroxy vitamin D are associated with elevated parathyroid hormone in healthy adolescent females. Osteoporos Int **16:**109–113.
44. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ 1997 Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int **7:**439–443.
45. Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B 1989 Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. N Engl J Med **321:**1777–1783.
46. McKenna MJ, Freaney R 1998 Secondary hyperparathyroidism in the elderly: Means to defining hypovitaminosis D. Osteoporos Int **8**(Suppl 2)**:**S3–S6.
47. Heaney RP, Dowell MS, Hale CA, Bendich A 2003 Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr **22:**142–146.
48. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE 1997 Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med **337:**670–676.
49. Kinyamu HK, Gallagher JC, Rafferty KA, Balhorn KE 1998 Dietary calcium and vitamin D intake in elderly women: Effect on serum parathyroid hormone and vitamin D metabolites. Am J Clin Nutr **67:**342–348.
50. Finestone A, Shlamkovitch N, Eldad A, Wosk J, Laor A, Danon YL, Milgrom C 1991 Risk factors for stress fractures among Israeli infantry recruits. Mil Med **156:**528–530.
51. Lehtonen-Veromaa M, Mottonen T, Irjala K, Karkkainen M, Lamberg-Allardt C, Hakola P, Viikari J 1999 Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. Eur J Clin Nutr **53:**746–751.

Address reprint requests to:
Juha-Petri S Ruohola, MD
Kiirunankuja 8
FIN-21210 Raisio, Finland
E-mail: j-p.ruohola@pp.inet.fi

Received in original form March 9, 2006; revised form May 11, 2006; accepted June 9, 2006.

An association of serum vitamin D <40 nmol/l with acute respiratory tract infection in young Finnish men

Ilkka Laaksi, Juha-Petri Ruohola, Pentti Tuohimaa, Anssi Auvinen, Riina Haataja, Harri Pihlajamäki, Timo Ylikomi

Department of Cell Biology, Medical School, 33014 University of Tampere Finland
Ilkka Laaksi
Senior Lecturer, General Practitioner

Garrison Hospital, Pori Brigade, Finnish Defence Forces, Säkylä, Finland
Juha-Petri Ruohola
Military Doctor

Department of Anatomy, Medical School, 33014 University of Tampere, Finland
Department of Clinical Chemistry, Tampere University Hospital, Finland
Pentti Tuohimaa
Professor

Department of Epidemiology, Tampere School of Public Health, University of Tampere Finland
Anssi Auvinen
Professor

Tampere School of Public Health, University of Tampere Finland
Riina Haataja
Reader

Department of Medical Services, Defence Staff, Finnish Defence Forces, Helsinki, Finland
Institute of Military Medicine, Central Military Hospital, Helsinki, Finland
Harri Pihlajamäki
Scientific Director

Department of Cell Biology, Medical School, 33014 University of Tampere, Finland
Department of Clinical Chemistry, Tampere University Hospital, Finland
Timo Ylikomi
Professor

The study was supported by grants from the Medical Research Fund of Tampere University Hospital and Finnish Defence Forces.

Correspondence to Ilkka Laaksi; Tel.: +358-3-2156661; Fax: +358-3-2156170; email: ilkka.laaksi@uta.fi

Abstract

Background

The effects of vitamin D to regulate bone mineralization are well documented. Recently, the action of vitamin D as a key link between Toll-like receptor activation and antibacterial responses in innate immunity was demonstrated. The data suggests that differences in ability of human populations to produce vitamin D may contribute to susceptibility to microbial infection.

Objective

The objective of the study was to explore whether an association exists between vitamin D insufficiency and acute respiratory tract infections in young Finnish men.

Design

800 young Finnish men serving in the military base in Finland were enrolled for this study. Their serum 25-OHD concentrations were measured in July 2002. They were followed for 6 months and the days of absence from duty due to respiratory infection were counted.

Results

The mean serum 25-OHD concentrations were 80.2 ± 29.3 nmol/l (n=756). Subjects with vitamin D insufficiency (<40 nmol/L, n=24) had significantly more absence from duty days due to respiratory infections (med: 4; q1-q3: 2-6) than controls (2; 0-4; n=628; incidence rate ratio 1.63; 95% CI 1.15-2.24; p=0.004). We found a significant association between serum 25-OHD concentration and frequency of previous physical exercise (p=0.004). We also

found significantly lower serum 25-OHD concentrations (72.8 ± 26.6 nmol/l; n=192) in subjects who smoked than in controls (82.9 ± 29.7 nmol/l; n=537), $p<0.001$.

Conclusions

Our findings suggest that consideration must be given to clinical trials of vitamin D supplementation to investigate whether it enhances immunity to microbial infections.

KEYWORDS: Vitamin D, respiratory infection, insufficiency, men, public health, 25-hydroxyvitamin D

Introduction

Vitamin D is produced in the skin through a photolysis reaction of 7-dehydrocholesterol induced by UVB radiation (290-315 nm). It is also a a substance which occurs naturally in foods. The metabolite formed in the skin and the vitamin D absorbed in the gut need to be hydroxylated in the liver to form 25-hydroxyvitamin D (25-OHD) and then in the kidney to form 1α, 25-dihydroxyvitamin D (1,25-$(OH)_2$D). (1) After these transformations, vitamin D is a biologically active substance, a hormone, chemically akin to steroid hormones. The main function of vitamin D in the body is to regulate the calcium and phosphorous homeostasis, a process essential for bone mineralization. (2) Vitamin D deficiency is known to lead to secondary hyperparathyroidism causing rickets in children and osteomalacia and osteoporosis in adults. (3)

To determine the vitamin D status, the serum concentration of 25-OHD, the major circulating form of the hormone, needs to be measured. (4) The seasonal variation of serum 25-OHD concentration reflects the importance of cutaneous synthesis as a source of vitamin D. Vitamin D insufficiency is usually determined by the parathyroid hormone (PTH) secretion, which increases when serum 25-OHD concentrations fall below 78-100 nmol/l. (5, 6) There is a growing scientific consensus that vitamin D insufficiency is reached for serum 25-OHD levels less than 80 nmol/L (7, 8).

In northern climates, diet is the most important source of vitamin D in the winter months, because exposure to sunlight is limited, resulting in inadequate endogenous production of

vitamin D. In Finland, with its geographical location between 60° and 70° N, vitamin D insufficiency has been reported to commonly occur in all age groups between October and March. (9, 10)

New, diverse roles of vitamin D have also been discovered, including its antiproliferative, differentiative, and apoptotic effects on prostate cancer cells *in vitro* and the effects on cancer invasion and angiogenesis.

Recently, Liu et al. demonstrated the action of vitamin D as a key link between Toll-like receptor (TLR) activation and antibacterial responses in innate immunity. They demonstrated a dose-dependent up-regulation of one known antimicrobial peptide (cathelicidin) in human monocytes. Addition of 1,25-$(OH)_2$D to primary human macrophages infected with Mycobacterium tuberculosis reduced the number of viable bacilli. Addition of either 25-OHD or TLR alone had no effect, but their simultaneous addition up-regulated cathelicidin mRNA. Furthermore, specific inhibition of 1-hydroxylase enzyme reduced this antimicrobial activity by 70%, suggesting that 1,25-$(OH)_2$D is the active form of vitamin D. They also found that the induction of cathelicidin mRNA was significantly lower in the presence of serum from African Americans, which contained less 25-OHD, than the serum from Caucasian individuals. However, supplementation of African American serum with 25-OHD restored the TLR induction of cathelicidin mRNA. (11)

Clarifying the role of vitamin D in relation to infections, such as acute respiratory tract infections, should deserve a high priority. (12) The aim of our study was to explore whether an association exists between vitamin D insufficiency and acute respiratory tract infections in young Finnish men.

Methods

Subjects

800 young Finnish men serving in the military base of Southwest Finland in July 2002 were enrolled for this study. When entering military service, they passed their entry medical examination as healthy. Conscripts taking vitamin D supplements or having travelled in sunny countries during the preceding 3 months were excluded from the study. The study population and the military environment conditions concerning physical activity, nutrition, clothing, living quarters, and exposure to sunlight were homogenous. The Ethics Committee of Pirkanmaa Hospital District approved the study. All volunteers signed a written informed consent.

Identification of respiratory infections

Medical records for all participants covering the first 6 months of military service were abstracted, and physician-diagnosed respiratory tract infections (sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, laryngitis) were recorded, and days of absence from duty due to respiratory tract infection were counted.

Background information

We used a structured questionnaire to elicit information on age, smoking, frequency of physical exercise before military service (hours per week), physician-diagnosed lactose intolerance, education (high school or higher), geographical location (Northern/Southern) and area of residence (urban/rural). The height and weight were measured to determine the body mass index (BMI).

Serum 25-OHD concentration

Blood specimens for serum 25-OHD concentration were drawn from 800 conscripts at entry into military service in the beginning of July 2002. Because of failed samples, incomplete follow-up data, and terminated military service, the total study population comprised 756 subjects. After coagulation at room temperature for 1 hour, the samples were centrifuged at 2000 G for 20 min at room temperature for serum separation. The serum samples were frozen and stored at –20ºC for later analysis. Total serum 25-OHD concentrations were measured with OCTEIA® enzymeimmunoassay by IDS (Immunodiagnostic Systems Inc, Fountain Hills, AZ, USA). According to the manufacturer, the cross-reactivities obtained for 25-OHD_3 and 25-OHD_2 are 100% and 75%, respectively. (13)

Statistics

Serum 25-OHD concentration was expressed as mean ± SD. Differences in means between independent samples were tested using Student's t-test or one-way analysis of variance (ANOVA). The number of absence from duty days due to respiratory infection

was expressed as median (med), and lower (q1) and upper quartile (q3), because the distribution was skew. Poisson regression analysis was used to explain this variable plus 0.5, because the variable included zeros. Overdispersion was estimated by the deviance divided by its degrees of freedom. Serum 25-OHD concentrations were categorised (<40 nmol/l and ≥40 nmol/l) for the regression analysis.

The limit for statistical significance was set equal to 0.05 for a two-sided test. We used SAS/Win (Version 8.2) for Poisson regression analysis and SPSS/Win (Version 11.0) for other data analysis.

Results

Vitamin D insufficiency was defined as a serum 25-OHD concentration of < 40 nmol/l. In July 2002, the mean serum 25-OHD concentrations were 80.2 ± 29.3 nmol/l (n=756), indicating vitamin D insufficiency in 3.6% of the subjects.

We found that subjects with vitamin D insufficiency (<40 nmol/l; n=24) had significantly more absence from duty days due to respiratory infections (med: 4; q1-q3: 2-6) than controls (2; 0-4; incidence rate ratio 1.63; 95% CI 1.15-2.24; n=628; p=0.004). The model was adjusted to smoking (n=169).

We also found a significant association between serum 25-OHD concentration and frequency of previous physical exercise (p=0.004). Subjects who exercised >5 h/wk had higher serum 25-OHD concentrations (85.8 ± 30.6 nmol/l) than subjects reporting no

physical exercise (71.3 ± 32.0 nmol/l; table). There was a non-significant association between frequency of physical exercise and number of absence from duty days due to respiratory infection (p=0.388; n=264).

Significantly lower serum 25-OHD concentrations (72.8 ± 26.6 nmol/l; n=192) were found in subjects who smoked than in controls (82.9 ± 29.7 nmol/l; n=537; table), p<0.001, and there was a near-significant association between smoking (n=169) and number of absence from duty days due to respiratory infection (incidence rate ratio 1.18; 95% CI 0.988-1.40; p=0.065; n=629).

There was a non-significant association between BMI and serum 25-OHD concentration, but no association was found between serum 25-OHD concentration and lactose intolerance. No differences were found in vitamin D status between subjects from Southern and Northern Finland, or between subjects living in urban or rural areas. Education level was not associated with vitamin D status (table).

Discussion

Our study contains several major findings. In July 2002, vitamin D insufficiency was identified in 3.6% of young Finnish men who were followed for 6 months during military service. Further, our findings demonstrated a significant, negative association of serum 25-OHD concentration with the number of absence from duty days due to physician-

diagnosed respiratory tract infection, a negative association with smoking, and a positive association with physical exercise.

All Finnish men are liable to complete a 6, 9, or 12-month compulsory military service between 18 and 29 years of age. Military service is voluntary for women. Each year, on average 26 500 male conscripts and 500 enlisted females undergo military training. Our study population of 756 conscripts represents homogeneity with respect to age and the conditions for physical activity, nutrition, clothing, living areas, and exposure to sunlight in the military environment. As the recruits live in close quarters, respiratory infections are common in garrisons.

The accuracy of the outcome data (respiratory infections identified) regarding absence from duty can be considered a strength of this study. Despite a relatively small number of men with low vitamin D concentrations, we were able to demonstrate a statistically significant effect on absence from duty due to infectious disease. Our results show that the statistical power was sufficient. Further, we were able to adjust for the effect of smoking on respiratory infections. Validity of our findings was also enhanced by the comparability of the conditions during the study for all subjects, unrelated to vitamin D concentration.

However, our study had several potential limitations. Since we obtained only one vitamin D measurement, the persistence of differences in vitamin D status during the study was not evaluated. Our study was not randomised and the validity requires comparability of

the groups with different levels of vitamin D. Although vitamin D was also correlated with the frequency of previous physical exercise, the latter was not associated with infections and therefore did not confound our results.

There is also strong evidence that to avoid secondary hyperparathyroidism and the resulting increase in calcium release and bone turnover rate, serum 25-OHD concentrations should be >100 nmol/l. (5, 9, 14, 15) In our study population, only 20.1% fulfilled that criterion in July 2002. The evidence that serum 25-OHD concentrations decrease somewhat with aging is suggestive of a potential risk for developing osteoporosis later in life. (9, 16)

According to our findings it seems likely that, in terms of adequate sunlight exposure, cutaneous synthesis of vitamin D is sufficient in Finland during summertime. In the winter months, however, vitamin D insufficiency in Finland is known to be very common. (9, 10) Since February 2003, following recommendations of the Ministry of Social Affairs and Health, vitamin D has been added to commercial milk and dairy products (0.5 µg/100 ml), and margarines (10 µg/100 g) in Finland. This fortification is part of the national health policy aimed at increasing vitamin D intake through diet to 280 IU/d. In our recent study, we showed that the mean wintertime serum 25-OHD concentrations increased by 50% after implementation of the vitamin D fortification recommendations. Correspondingly, the prevalence of vitamin D insufficiency (serum 25-OHD <40 nmol/l) decreased by 50%, from 78% to 35%. In spite of these efforts, however, vitamin D concentrations remain low in a third of young Finnish men during

the winter months.(17) It should be noted that the present study was conducted before the fortification recommendations were implemented.

Based on our finding that a low vitamin D status at initial entry into military service and subsequent respiratory infections are statistically significantly related, it seems evident that vitamin D insufficiency contributes to proneness to these diseases.

Taking into account the geographical position of Finland, extending from the 60^{th} to the 70^{th} northern parallel, we expected to see regional differences in vitamin D levels of the subjects. However, we found no statistically significant differences in serum 25-OHD concentrations between subjects from Northern or Southern Finland.

Our findings contribute to the diversity of consequences already known to result from vitamin D insufficiency and recognised as carrying important global public health implications. In the context of immune function, clarification of the role of vitamin D in relation to infections, such as acute respiratory tract infections, represents a high priority for future research. Furthermore, consideration must be given to clinical trials of vitamin D supplementation to investigate whether it enhances immunity to microbial infections.

Acknowledgements

We thank the conscripts who agreed to participate in the study.

I Laaksi was the principal investigator, conceived the idea for the study, prepared the study protocol and drafted the manuscript. I Laaksi, J Ruohola and H Pihlajamäki participated in data collection. I Laaksi, J Ruohola, H Pihlajamäki, P Tuohimaa and T Ylikomi coordinated the study and obtained funding. R Haataja and A Auvinen provided statistical expertise. T Ylikomi, H Pihlajamäki, J Ruohola, R Haataja and A Auvinen participated in writing the draft and making final corrections to this report. H Pihlajamäki and T Ylikomi supervised the study.

We have no conflict of interest to declare.

We wish to thank Mrs Hilkka Mäkinen for excellent technical assistance.

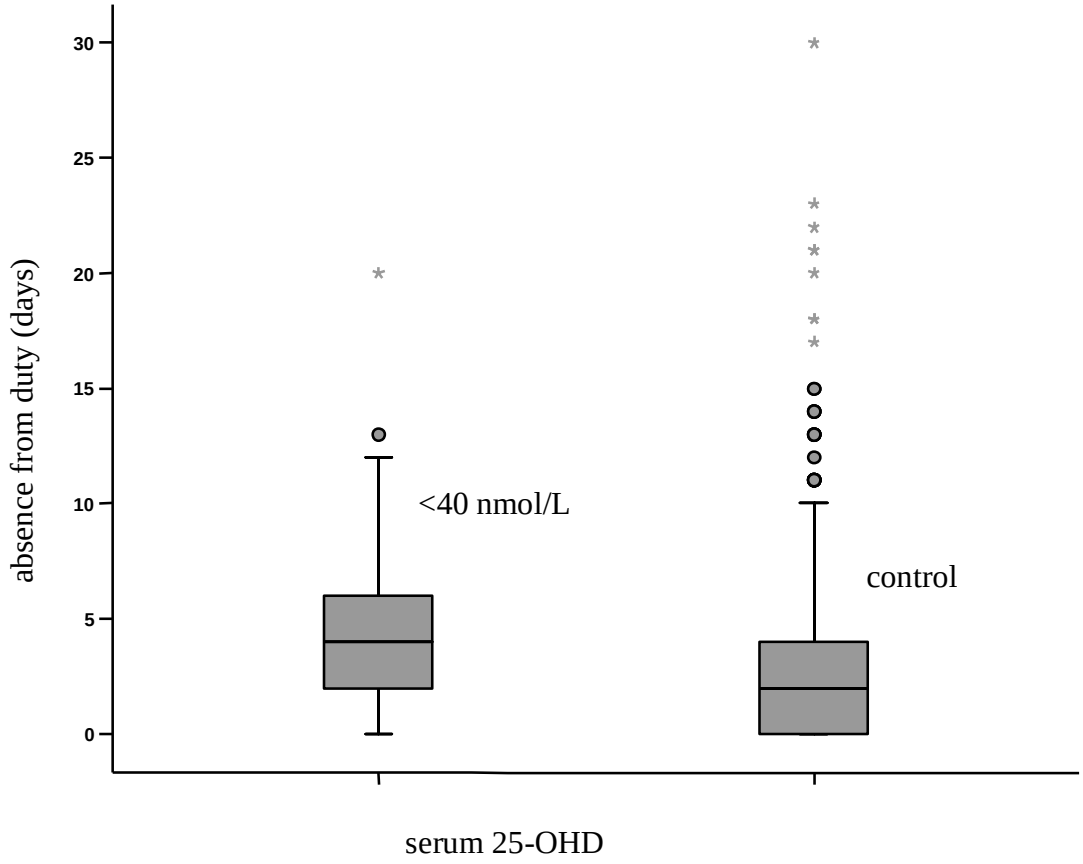
References

1. Holick MF, Uskokovic M, Henley JW, MacLaughlin J, Holick SA, Potts JT, Jr. The photoproduction of 1 alpha,25-dihydroxyvitamin D3 in skin: an approach to the therapy of vitamin-D-resistant syndromes. N Engl J Med 1980;303:349-54.
2. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem 2003;88:296-307.
3. Compston JE. Vitamin D deficiency: time for action. Evidence supports routine supplementation for elderly people and others at risk. Bmj 1998;317:1466-7.
4. Utiger RD. The need for more vitamin D. N Engl J Med 1998;338:828-9.
5. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7:439-43.
6. McKenna MJ, Freaney R. Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. Osteoporos Int 1998;8 Suppl 2:S3-6.
7. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713-6.
8. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.
9. Valimaki VV, Alfthan H, Lehmuskallio E, et al. Vitamin D status as a determinant of peak bone mass in young Finnish men. J Clin Endocrinol Metab 2004;89:76-80.
10. Lamberg-Allardt CJ, Outila TA, Karkkainen MU, Rita HJ, Valsta LM. Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? J Bone Miner Res 2001;16:2066-73.
11. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770-3.
12. Raiten DJ, Picciano MF. Vitamin D and health in the 21st century: bone and beyond. Executive summary. Am J Clin Nutr 2004;80:1673S-7S.
13. Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme. Clin Chem 2004;50:2195-7.
14. Kinyamu HK, Gallagher JC, Rafferty KA, Balhorn KE. Dietary calcium and vitamin D intake in elderly women: effect on serum parathyroid hormone and vitamin D metabolites. Am J Clin Nutr 1998;67:342-8.
15. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997;337:670-6.
16. Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporos Int 1999;9:394-7.
17. Laaksi IT, Ruohola JP, Ylikomi TJ, et al. Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. Eur J Clin Nutr 2006.

Table

	25-OHD concentration (nmol/l)		
	n	mean ± SD	p-value
BMI			0.092
< 20.0	63	76.5 ± 31.0	
20.0 – 24.9	345	83.0 ± 30.9	
25.0 – 30.0	127	79.7 ± 25.8	
> 30	29	71.4 ± 21.8	
Smoking			<0.001
yes	192	72.8 ± 26.6	
no	537	82.9 ± 29.7	
Alcohol use			0.306
< once a month	39	74.2 ± 28.1	
once a month	99	82.3 ± 35.6	
> once a month	152	77.6 ± 27.5	
Frequency of physical exercise			0.004
>5 h/wk	99	85.8 ± 30.6	
≤ 5 h/wk	96	79.6 ± 27.6	
no physical exercise	92	71.3 ± 32.0	
Diagnosed lactose intolerance			0.474
yes	30	82.9 ± 33.5	
no	255	78.7 ± 30.4	
Geographical location of residence in Finland			0.632
Northern	35	81.1 ± 28.4	
Southern	255	78.4 ± 30.9	
Area of residence			0.275
urban	208	80.0 ± 30.1	
rural	82	75.6 ± 31.6	
Education level			0.716
no high school	127	79.3 ± 32.6	
high school or higher	154	78.0 ± 29.4	

Fig. Subjects with serum 25-OHD < 40 nmol/L (n=24) had significantly more absence from duty days due to respiratory infections than controls (n=604, p=0.004). The model was adjusted to smoking.



Vitamin D supplementation for the prevention of acute respiratory tract infections; a randomized double-blinded trial in young Finnish men

Ilkka Laaksi, Juha-Petri Ruohola, Ville Mattila, Anssi Auvinen, Timo Ylikomi, Harri Pihlajamäki

Department of Cell Biology, Medical School, 33014 University of Tampere Finland
Ilkka Laaksi
Lecturer, Medical Doctor

Finnish Defence Forces, Finland
Juha-Petri Ruohola
Military Doctor, Researcher

Research Department, Centre for Military Medicine, Helsinki, Finland
Ville Mattila
Adjunct Professor

Department of Epidemiology, Tampere School of Public Health, University of Tampere, Finland
Anssi Auvinen
Professor

Department of Cell Biology, Medical School, 33014 University of Tampere, Finland
Department of Clinical Chemistry, Tampere University Hospital, Finland
Timo Ylikomi
Professor

Research Department, Centre for Military Medicine, Helsinki, Finland

Harri Pihlajamäki
Adjunct Professor, Scientific Director

The study was supported by grant from the Medical Research Fund of Finnish Defence Forces.

Correspondence to Ilkka Laaksi; Tel.: +358-3-35516661; Fax: +358-3-35516170; email: ilkka.laaksi@uta.fi

The manuscript consists of 2837 words, 24 pages, 2 tables and 1 figure.

Introduction

Vitamin D is formed in the skin from 7-dehydrocholesterol after activation induced by UVB-radiation (290-315 nm). Vitamin D is the precursor of the hormone 1,25$(OH)_2$D, which is formed in two hydroxylation reactions, first to 25-hydroxyvitamin D [25(OH)D] in the liver and then to 1,25-$(OH)_2$D in the kidneys or target organs.[1] Vitamin D regulates the calcium and phosphate balance as well as bone mineralization,[2] and its deficiency leads to secondary hyperparathyroidism, causing rickets in children and osteoporosis and osteomalacia in adults.[3, 4]

Vitamin D status is determined by measuring the serum concentration of 25(OH)D, the major circulating form of the hormone.[5] The emerging consensus is that vitamin D insufficiency be defined as serum 25(OH)D levels of less than 80 nmol/L.[6, 7] Diet is the most important source of vitamin D in northern latitudes during the wintertime, as sunlight exposure during this time is inadequate for inducing the endogenous production of vitamin D. Vitamin D deficiency is common in all age groups in Finland between October and March.[8] As a public health policy, vitamin D fortification of liquid milk products (0.5 µg/dL) and margarines (10 µg/100 g) has been implemented in Finland since February 2003.

Vitamin D regulates gene expression through binding with vitamin D receptors (VDR), as active vitamin D binds to the VDR, which dimerizes with the retinoic X receptor. The vitamin D/VDR complex binds to vitamin D-responsive elements (VDRE) inside the promoter regions of vitamin D-responsive genes. Nuclear receptor coactivator proteins enhance this transcriptional activation. VDR modulates the expression of genes that are involved in immunity.[9, 10]

Toll-like receptors (TLRs) monitor the host for the presence of pathogens. TLR stimulation by pathogen lipopeptides leads to the production of antimicrobial peptides.[11] Moreover, TLR activation of human macrophages upregulates VDR expression and the vitamin D-1 hydroxylase genes, which enhances the induction of the cathelicidin. Adenosine monophosphate is upregulated by vitamin D in human monocytes in a dose-dependent manner. Liu et al (2006)

reported that the induction of cathelicidin mRNA was significantly lower in the presence of serum from African Americans, which contains less 25(OH)D than does the serum from Caucasians. [12]

Respiratory infection leads to increased activation of vitamin D and increased levels of cathelicidin mRNA. Specifically, respiratory epithelial cells activate vitamin D and create a microenvironment with high levels of the active form of the vitamin. This local vitamin D activation might be an important component of host defense because it has downstream effects, including upregulation of the cathelicidin antimicrobial peptide gene, which is an important component of innate immunity in the lungs.[13]

Clinically, antimicrobial peptides inhibit invasive pneumococcal disease, meningococcal disease, and group A streptococcal disease [14, 15]. Vitamin D deficiency seems to be a risk factor for severe respiratory infection in children under the age of 5 years.[16]

Vitamin D production in the skin is seasonal; vitamin D deficiency is common in the winter and $1,25(OH)_2D$ stimulates the expression of antimicrobial peptides in epithelial cells lining the respiratory tract, thus protecting the lung from infection. It has been proposed that vitamin D deficiency is a 'seasonal stimulus', which explains the remarkable seasonality of epidemic influenza. [17]

Our earlier observational study in 754 young Finnish men demonstrated a significant negative association between serum 25(OH)D levels and the number of days absent from daily duty due to acute respiratory tract infection.[18] The present study was a blinded, placebo-controlled randomized trial whose primary outcome was to determine whether vitamin D supplementation decreases the number of days absent from duty due to acute respiratory tract infection.

Subjects and Methods

Trial design

This placebo-controlled double-blinded study comprised 164 voluntary young Finnish men (18-28 years) undergoing compulsory periodic military training as conscripts in an infantry unit comprising 400 men in the Finnish Defence Forces. The subjects represented the general conscript population of the Finnish Defence Forces. Inclusion criteria were no regular medication, and passing the entry medical examination as healthy. Exclusion criteria were the use of supplementary vitamin D, multivitamins, and cod liver oil. Of a total of 400 men entering the unit, 164 men (41%) volunteered to participate in the study and met the inclusion criteria. The subjects were randomly assigned to the intervention group, which received 400 IU (10 µg, n=80) vitamin D_3 (Minisun®, Verman) daily, or the control group (n=84), which received placebo (Pharmia, a capsule identical in size and form to the active preparation). Random allocation was performed using computer-generated random numbers. The conditions related to physical activity, nutrition, clothing, accommodation, and exposure to sunlight were homogeneous during their military service. The trial was performed from October to March in Pori Brigade, in southwestern Finland. This study was approved by the Ethics Committee of Tampere University Hospital, Finland. Participation was voluntary and written informed consent was obtained from all participants. The study was registered in ClinicalTrials.gov at NCT00973583.

Identification of respiratory infections

Medical records for all participants covering the first 6 months of military service were reviewed and any diagnosed acute respiratory tract infection (i.e., sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, and laryngitis) was recorded. The main outcome variable was the number of days absent from duty due to acute respiratory tract infection. Secondary outcomes were self-reported symptoms of acute respiratory tract infection (cough, runny nose, sore throat,

fever, or common cold symptoms) and hospitalization due to acute respiratory tract infection. The symptoms were evaluated four times during the study. The physicians and other personnel treating patients in garrisons were blinded to treatment allocation. All acute respiratory tract infections were treated in the garrison hospital. The shared environment and tasks, as well as the closed community of the military troops with a uniform setting, homogeneity in terms of source of infection and large population in close daily contact makes this group susceptible to minor epidemics, which increases the probability of infection and therefore increased the statistical power of the study.

After randomization, blood samples were drawn from 73 subjects at the beginning of the study in October 2005 and again from 108 subjects in March 2006 to determine the serum 25(OH)D concentrations. The samples were coagulated at room temperature for 1 hour and centrifuged at 2000g for 20 min at room temperature for serum separation. Serum samples were then frozen and stored at –20ºC for later analysis. Total serum 25(OH)D concentrations were measured using an OCTEIA® enzyme immunoassay kit (Immunodiagnostic Systems Inc, Newcastle, UK).

Plasma parathyroid hormone (PTH) concentrations were measured by electrochemiluminescence (Elecsys PTH Kit, Roche Diagnostics, Mannheim, Germany) in 104 randomly chosen subjects at the end of the study. Elecsys PTH CalSet (Roche Diagnostics) was used for calibration.

Statistical analysis

We aimed to maximize the power by recruiting all voluntary conscripts for the study (i.e., we did not sample the target population). Hence, formal sample size calculations were not performed prior to the study. Our primary analysis included all randomized subjects in accordance with the intention-to-treat principle. Differences between the groups in continuous variables were tested using the Mann-Whitney U-test. Chi-square tests were used to assess categorical data. We set a two-sided p value of less than 0.05 as the alpha criterion. Hazard ratio was calculated by Cox's regression analysis; end-point of the follow-up was the first infection, with censoring at premature release from duty, or at the end of the study after the 6-month follow-up. Cox's

regression analysis was adjusted for influenza vaccination and smoking at baseline. The frequency of missing variables at the baseline varied from 2% to 6%, but for smoking it was 20%. Altogether 60 subjects dropped out of the study by the endpoint, with no specific reason given (Figure). Data analysis was performed with SPSS for Windows (version 15.0.1; SPSS Inc., Chicago, IL).

Results

In October 2005, at the beginning of the study, there was no difference in serum 25(OH)D concentrations between the intervention (78.7 ± 14.9 nmol/L, n=29) and placebo (74.4 ± 20.8 nmol/L, n=44) groups (p=0.35). Other characteristics were also comparable between the groups at baseline, though both smoking and influenza vaccination were slightly more common in the placebo group (Table 1).

In March 2006, after daily supplementation with 400 IU vitamin D or placebo for 6 months, the mean serum 25(OH)D concentrations were 71.6 ± 22.9 nmol/L (n=58) in the intervention group and 51.3 ± 15.5 nmol/L (n=50) in the placebo group (p<0.001).

The main outcome variable, number of days absent from duty due to respiratory tract infection, did not differ between groups. Mean number of days absent was 2.2 (SD 3.2) in the intervention group and 3.0 (SD 4.0) in the placebo group (p=0.096). There was an effect during the first 6 weeks of the study, with a mean of 0.7 (SD 2.1) days of absence in the intervention group and a mean of 1.4 (SD 2.6) days absent in the placebo group (p=0.060). After the first 6 weeks, there tended to be no difference between groups (Table 2). Nevertheless, the proportion of men remaining healthy throughout the 6-month study period was greater in the intervention group (51.3%, n=41) than in the placebo group (35.7%, n=30, p=0.045). In a Cox regression analysis with adjustments for smoking and influenza vaccination, the adjusted hazard ratio (HR) for absence from duty due to respiratory tract infection was lower in the intervention group (HR 0.71; 95% CI: 0.43-1.15). The number needed to treat calculated from the proportion of men without any days absent from duty was 6.4 (95% CI: 3-257). Self-reported cough (65% in the intervention group vs. 57% in the placebo group, p=0.30), runny nose (74% vs. 75%, p=0.86), sore throat (48% vs. 45%, p=0.77), fever (31% vs. 38%, p=0.36), and common cold symptoms (56% vs. 52%, p=0.40) did not differ between the groups. The mean number of hospital days was 0.31 (SD 1.21) per subject in the intervention group and 0.90 (SD 2.22) in the placebo group (p=0.06). Plasma PTH concentrations did not significantly differ between the intervention 4.3 ± 1.3 ng/L (n=58) and placebo 4.4 ± 1.4 ng/L (n=50) groups (p=0.55).

Two subjects in the intervention group reported nausea, stomachache, and diarrhea. One subject in the placebo group dropped out of the study due to a facial rash acquired during the study.

Discussion

The present placebo-controlled double-blinded study of 164 young Finnish men provides some evidence for a preventive effect of vitamin D supplementation against respiratory tract infection. The Cox's regression analysis indicated that the hazard ratio for absence from duty due to respiratory tract infection was lower in the vitamin D supplementation group than in the controls. The number of days absent was slightly lower in the vitamin D supplementation group and the proportion without any days absent was slightly higher in the vitamin D supplementation group compared with controls. The number needed to treat calculated from the proportion of men without any days absent from duty was as low as 6.4, but there was a very wide confidence interval (3-257). Further, subjects receiving 400 IU vitamin D daily had fewer days of absence due to respiratory infection during the first 6 weeks of follow-up.

This study has some limitations. The primary end-point (number of days absent from duty) did not differ significantly between groups, and an effect emerged only in the secondary outcome measures. A formal a priori power calculation could not be performed due to lack of a defined clinically meaningful difference in infection rates. In addition, the power of the study was limited by the number of drop-outs.

The original sample size was sufficient to show a difference between the mean number of days absent of 1.9 (SD 2.9) versus 3.0 (SD 4.0), assuming no drop-outs. The observed effect was 72% of that size, similar to the drop-out rate in the intervention group. Thus, with perfect compliance we might have been able to demonstrate an effect with a study group of the original size. Nonetheless, the difference in the mean number of days absent between the trial arms would have been statistically significant had a one-sided p-value been used in accordance with the direction of the study hypothesis.

In a recent 6-month double-blinded vitamin D intervention study of Finnish men aged 21 to 49 years, a winter-time elevation of serum PTH was inhibited by vitamin D supplementation (800

IU/d). In our study, vitamin D supplementation with 400 IU/d had no significant effect on plasma PTH concentrations. [19]

Serum 25(OH)D concentrations of the conscripts were insufficient at baseline in October (76 nmol/L). In March, after 6 months of vitamin D supplementation, the study group still had an insufficient vitamin D status (72 nmol/l) and the subjects in the placebo group had a vitamin D deficiency (51 nmol/L, $p<0.001$). Furthermore, serum 25(OH)D levels were greater than 80 nmol/L in only 8% of the placebo group compared to 29% in the intervention group. Based on the average consumption of milk and margarine in the Finnish Defence Forces, these young men typically receive 7 μg (280 IU) of vitamin D daily from vitamin D-fortified products. The results of the study indicated that additional supplementation with 400 IU/d of vitamin D is not sufficient to maintain an adequate vitamin D level throughout the wintertime.[8]

All Finnish men must complete 6, 9, or 12 months of compulsory military service between 18 and 29 years of age. Military service is voluntary for women. Each year, an average of 26,500 male conscripts and 500 enlisted females undergo military training. Our study population of 164 conscripts is homogeneous with respect to age and conditions, including physical activity, nutrition, clothing, living areas, and exposure to sunlight in the military environment. As the conscripts live in close quarters, respiratory infections are common in garrisons, thereby offering an optimal setting for this kind of study. The homogeneity of our study setting and population is exceptional, and is a strength of the study. The completeness of the outcome data (respiratory infections identified) regarding absence from duty is also a strength.

In a study of 25 newborns with acute lower respiratory infection (ALRI) and 15 healthy newborns as controls, mean serum 25(OH)D concentrations were lower in the ALRI group ($p=0.011$), suggesting that vitamin D deficiency might be a risk factor for developing ALRI.[20] Another study of 56 young children hospitalized with ALRI and 64 children without a history of ALRI reported an association of VDR polymorphism with a 7-fold higher risk for ALRI.[21]

On the other hand, in another study of patients ranging in age from 1 to 25 months who were admitted to the hospital with uncomplicated ALRI and healthy similarly-aged patients without a

history of hospitalization for ALRI as a control group, serum 25(OH)D concentrations were equivalent between groups and there was no case-control difference in the prevalence of vitamin D deficiency.[22]

Interestingly, a recent study of the Third National Health and Nutrition Examination Survey including 18,883 participants 12 years of age and older reported that lower 25(OH)D levels were independently associated with recent upper respiratory tract infections (URTI). The median serum 25(OH)D level was 73 nmol/L, and 19% of participants reported a recent URTI. Upper respiratory tract infection was reported by 24% of the participants with 25(OH)D levels under 25 nmol/L, 20% with levels of 25 to 75 nmol/L, and 17% with levels of 75 nmol/L or more ($P < 0.001$). The relative risk for URTI was 1.4 times higher in participants with serum 25(OH)D levels under 25 nmol/L (OR 1.36; 95% CI, 1.01-1.84) and 1.2 times higher in those with serum 25(OH)D levels of 25 to 75 nmol/L (OR 1.24; 95% CI, 1.07-1.43). Further, the association seemed to be stronger in individuals with asthma and chronic obstructive pulmonary disease (OR, 5.67 and 2.26, respectively).[23]

A recent study of vitamin D supplementation showed no benefit in decreasing the incidence or severity of symptomatic upper respiratory infection (URI) during the winter season. In that study, 162 adults were randomized to receive 50 µg (2000 IU) of vitamin D_3 daily or placebo for 12 weeks. The subjects filled out a bi-weekly questionnaire to record the incidence or severity of URI. There was no difference in either the incidence of URIs ($p=0.57$) or the duration or severity of URI symptoms ($p=0.86$) between vitamin D and placebo groups. In addition, in that study the mean 25(OH)D level at baseline was similar between the vitamin D and placebo groups (64.3 ± 25.4 and 63.0+/-25.8 nmol/l, respectively). It is noteworthy that at 12 weeks, 25(OH)D levels had significantly increased to 88.5+/-23.2 nmol/l in the vitamin D group, but there was no decrease in vitamin D levels in the placebo group.[24]

Finally, both our earlier findings that a low vitamin D status at initial entry into military service and subsequent respiratory infections are significantly related [18] and the results of the present study provide some evidence for vitamin D supplementation toward the prevention of respiratory infections. Following the recommendation for vitamin D use by the Finnish Ministry of Social

Affairs and Health of 400 IU of vitamin D daily was used in the study. Randomized controlled trials with higher doses and larger populations are warranted to explore the preventive effect of vitamin D supplementation on acute respiratory tract infection.

Acknowledgements

The funding source had no role in the collection, analyses, interpretation of data, writing of the report, or decision to publish. The authors had full access to all the data in the study and had final responsibility for the decision to submit the study for publication.

I Laaksi, the principal investigator, conceived the idea for the study and drafted the manuscript. I Laaksi, J Ruohola, and H Pihlajamäki participated in the data collection. I Laaksi, J Ruohola, H Pihlajamäki, A Auvinen, and T Ylikomi prepared the study protocol and coordinated the study. I Laaksi, T Ylikomi and H Pihlajamäki obtained funding. V Mattila and A Auvinen provided statistical expertise. T Ylikomi and H Pihlajamäki supervised the study.

We have no conflict of interest to declare.

We thank the conscripts who agreed to participate in the study. We thank Mrs. Hilkka Mäkinen for excellent technical assistance and Jaakko Pitkäjärvi, MD for participating in the data collection.

Table 1. Patient characteristics at baseline

Characteristic	Total (n=164)	Vitamin D supplementation group (n=80)	Placebo group (n=84)
Height*	179.5 (5.9)	180.4 (5.8)	178.8 (5.9)
Weight*	75.3 (8.8)	75.5 (9.0)	75.2 (8.5)
Body mass index (kg/m^2)*	23.4 (2.7)	23.3 (2.6)	23.6 (2.8)
Daily smoking (%)	35 (21.3)	15 (24.0)	20 (29.9)
Influenza vaccination (%)	122 (74.8)	57 (71.3)	65 (77.4)

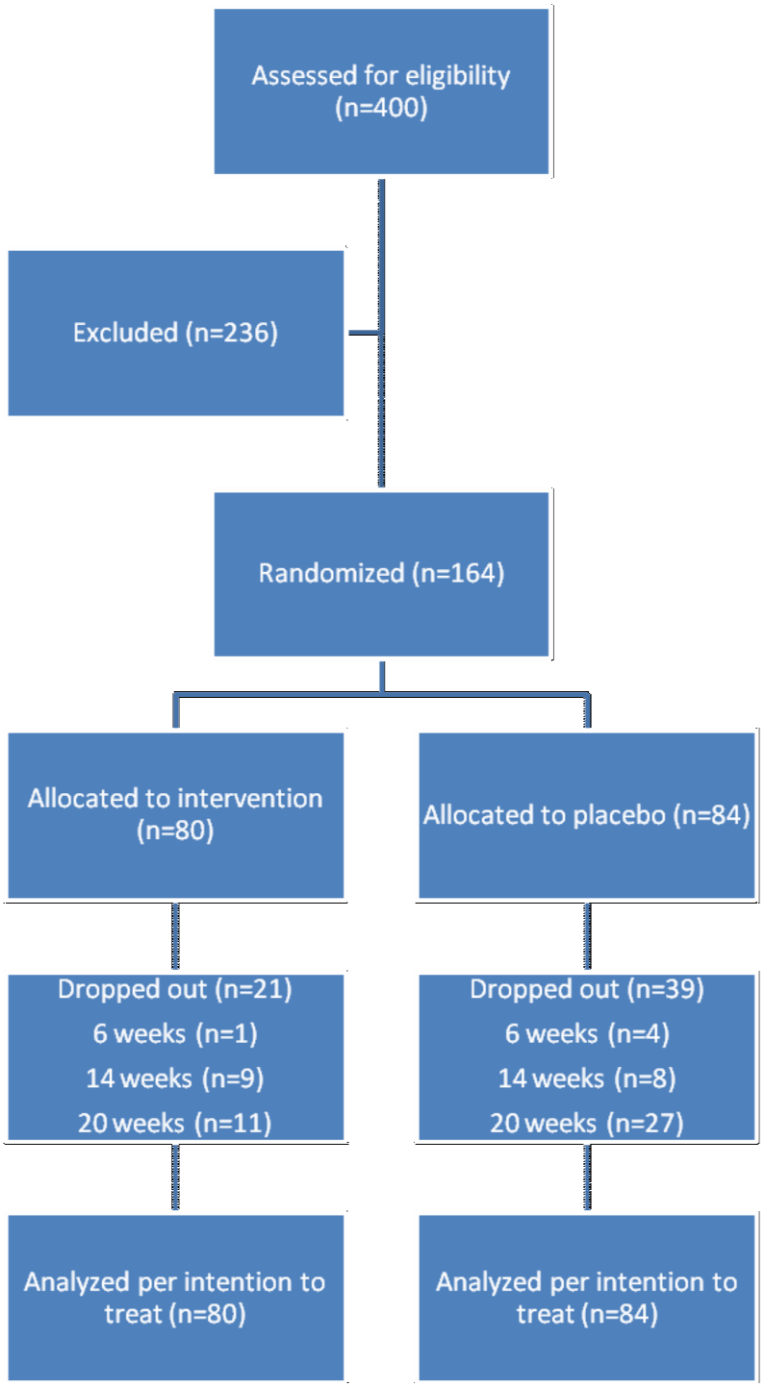
* The values are given as the means and standard deviations.

Table 2. Study events

Event	Total (n=164)	Vitamin D arm (n=80)	Placebo arm n=84)	p-value
Days absent from duty, mean (SD)	2.6 (3.6)	2.2 (3.2)	3.0 (4.0)	0.096
1 to 6 weeks	1.1 (2.4)	0.7 (2.1)	1.4 (2.6)	0.060
7 to 14 weeks	0.7 (1.8)	0.7 (1.4)	0.8 (2.1)	0.903
15 to 20 weeks	0.5 (1.0)	0.4 (1.0)	0.5 (1.1)	0.120
21 to 24 weeks	0.4 (1.5)	0.4 (1.8)	0.3 (1.1)	0.311
No days absent from duty**, number (%)	71 (43.3)	41 (51.3)	30 (35.7)	0.045
1 to 6 weeks	121 (73.8)	64 (80.0)	57 (67.9)	0.077
7 to 14 weeks	121 (76.1)	61 (77.2)	60 (75.0)	0.845
15 to 20 weeks	106 (75.7)	58 (82.9)	50 (69.4)	0.077
21 to 24 weeks	84 (80.7)	47 (79.7)	37 (82.2)	0.284
Self-reported symptoms, number (%)				
Cough	100 (61.0)	52 (65.0)	48 (57.1)	0.303
Runny nose	122 (74.4)	59 (73.8)	63 (75.0)	0.855
Sore throat	76 (46.3)	38 (47.5)	38 (45.2)	0.772
Fever	57 (34.8)	25 (31.3)	32 (38.1)	0.357
Common cold symptoms	89 (54.3)	45 (56.3)	44 (52.4)	0.619
Hospitalization due to respiratory tract infection, number (%)	9 (5.5)	3 (3.8)	6 (7.1)	0.396
Length of hospital stay, mean (SD)	0.2 (1.1)	0.2 (0.8)	0.3 (1.3)	0.338

**Proportions are calculated from subjects at the time of the study (figure).

Figure Patient flow chart



References

1. Holick MF, Uskokovic M, Henley JW, MacLaughlin J, Holick SA, Potts JT, Jr. The photoproduction of 1 alpha,25-dihydroxyvitamin D3 in skin: an approach to the therapy of vitamin-D-resistant syndromes. N Engl J Med 1980; 303:349-54.

2. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem 2003; 88:296-307.

3. Compston JE. Vitamin D deficiency: time for action. Evidence supports routine supplementation for elderly people and others at risk. BMJ 1998; 317:1466-7.

4. Ruohola JP, Laaksi I, Ylikomi T, et al. Association between serum 25(OH)D concentrations and bone stress fractures in Finnish young men. J Bone Miner Res 2006; 21:1483-8.

5. Utiger RD. The need for more vitamin D. N Engl J Med 1998; 338:828-9.

6. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005; 16:713-6.

7. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; 84:18-28.

8. Laaksi IT, Ruohola JP, Ylikomi TJ, et al. Vitamin D fortification as public health policy: significant improvement in vitamin D status in young Finnish men. Eur J Clin Nutr 2006 60:1035-8.

9. Baker AR, McDonnell DP, Hughes M, et al. Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci U S A 1988; 85:3294-8.

10. Rachez C, Freedman LP. Mechanisms of gene regulation by vitamin D(3) receptor: a network of coactivator interactions. Gene 2000; 246:9-21.

11. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004; 173:2909-12.

12. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006; 311:1770-3.

13. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol 2008; 181:7090-9.

14. Vlaminckx BJ, van Pelt W, Schouls LM, et al. Long-term surveillance of invasive group A streptococcal disease in The Netherlands, 1994-2003. Clin Microbiol Infect 2005; 11:226-31.
15. Lee HY, Andalibi A, Webster P, et al. Antimicrobial activity of innate immune molecules against Streptococcus pneumoniae, Moraxella catarrhalis and nontypeable Haemophilus influenzae. BMC Infect Dis 2004; 4:12.
16. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. Eur J Clin Nutr 2004; 58:563-7.
17. Cannell JJ, Vieth R, Umhau JC, et al. Epidemic influenza and vitamin D. Epidemiol Infect 2006; 134:1129-40.
18. Laaksi I, Ruohola JP, Tuohimaa P, et al. An association of serum vitamin D concentrations <40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 2007; 86:714-7.
19. Viljakainen HT, Vaisanen M, Kemi V, et al. Wintertime Vitamin D supplementation inhibits seasonal variation of calcitropic hormones and maintains bone turnover in healthy men. J Bone Miner Res 2009; 24:346-52.
20. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. Eur J Clin Nutr 2009; 63:473-7.
21. Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. J Infect Dis 2008; 197:676-80.
22. Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. Eur J Clin Nutr 2009; 63:297-9.
23. Ginde AA, Mansbach JM, Camargo CA, Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169:384-90.
24. Li-Ng M, Aloia JF, Pollack S, et al. A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. Epidemiol Infect 2009; 137:1396-404.