

Draft Vitamin D and Health report

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1. Introduction

Background

1. Vitamin D is synthesised in the skin by the action of sunlight. Skin synthesis is the main source of vitamin D for most people; dietary sources are essential when exposure to sunlight containing the appropriate wavelength is limited. The Committee on Medical Aspects of Food and Nutrition Policy (COMA), which set Dietary Reference Values (DRVs) for vitamin D in 1991 (DH¹, 1991), did not set a Reference Nutrient Intake (RNI²) for groups in the population considered to receive adequate sunlight exposure. It was assumed that, for most people, the amount of vitamin D produced by exposure to summer sunlight would produce enough vitamin D for their needs during winter.
2. Current UK government advice is that no dietary intake of vitamin D is necessary for individuals living a 'normal lifestyle'. Only certain groups of the population, who are at risk of vitamin D deficiency, are advised to take a daily supplement: pregnant and breastfeeding women (10 µg), infants and children aged under 4 years (7-8.5 µg); adults over 65 years (10 µg); those with limited exposure to the sun (e.g., if they cover their skin for cultural reasons or are housebound) (10 µg) and people of Asian origin (10 µg). The DRVs for vitamin D were reviewed and endorsed by COMA in 1998.
3. Although the current recommendations for vitamin D are based on bone health, it is suggested that vitamin D may have a role in other health outcomes including reducing the risk of cancer, cardiovascular disease (CVD), infectious diseases and autoimmune diseases.
4. The evidence on vitamin D and health was previously considered by the Scientific Advisory Committee on Nutrition (SACN) in 2007 in its position statement '*Update on Vitamin D*'. At that time, SACN concluded that there was insufficient evidence to reconsider the existing COMA DRVs for vitamin D and that the evidence on the relationship between vitamin D status and chronic disease, other than the metabolic bone diseases rickets and osteomalacia, was insufficient to draw conclusions.
5. In October 2010, SACN agreed to review the data on vitamin D because a significant amount of new evidence had accumulated since its previous considerations including: results from research commissioned by the Food Standards Agency (FSA); a detailed report published by the Institute of Medicine (IOM) in the United States (US) on *Dietary Reference Intakes for Calcium and Vitamin D* (2011); and numerous studies on vitamin D and various health outcomes.

Terms of Reference

6. The *SACN Working Group on Vitamin D* was established in 2011 to consider whether the current DRVs for vitamin D intake set by COMA in 1991 were still appropriate to ensure vitamin D adequacy of the UK population in the context of current lifestyles and public health advice (e.g., to stay out of sun and to wear sunscreen).
7. The terms of reference of the *SACN Working Group on Vitamin D* were: *to review the Dietary Reference Values for vitamin D and make recommendations.*
8. This required a risk assessment of the vitamin D status of the UK population and consideration of the:
 - biochemical indicators of vitamin D status and the validity of the values used to assess risk of deficiency and excess;
 - association between vitamin D status and various health outcomes at different life stages and in different population groups and the effects of biological modifiers;

¹ Department of Health

² The amount of a nutrient that is sufficient to meet the needs of 97.5% of the population.

- contribution of cutaneous vitamin D synthesis to vitamin D status in the UK taking account of the effects of modifiers of skin exposure to sunlight; the risks of skin damage and other adverse health outcomes associated with sunlight exposure;
- potential adverse effects of high vitamin D intakes;
- relative contributions made by dietary vitamin D intake (from natural food sources, fortified foods and supplements) and cutaneous vitamin D synthesis to the vitamin D status of the UK population.

Methodology

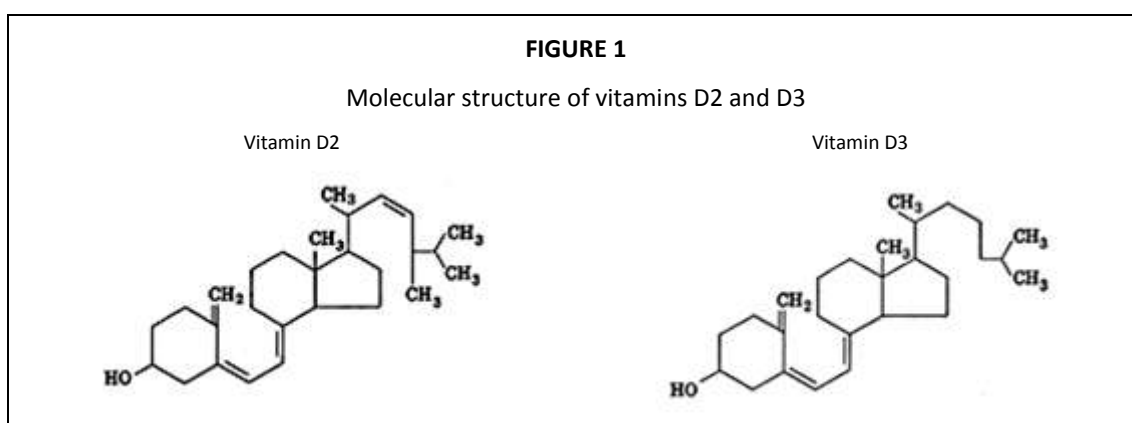
9. In considering the evidence on vitamin D and health outcomes, the preference was for randomised controlled trials (RCTs) and then prospective cohort studies. Other types of human studies (e.g., case-control, cross-sectional, case-reports) were considered when RCTs or prospective cohort studies were not available. Where possible, any dietary recommendations were based on data from RCTs.
10. Data from the National Diet and Nutrition Survey, the Health Survey for England, the Low Income Diet and Nutrition Survey, the UK Diet and Nutrition Survey of Infants and Young Children and the Scottish Health Survey were used to assess the vitamin D status of the UK population.
11. The IOM report on *Dietary Reference Intakes for Calcium and Vitamin D* (2011) provided an important and comprehensive database and a useful reference resource for consideration of the evidence on vitamin D and health outcomes. The IOM report was informed by two systematic reviews of the evidence (Cranney *et al*, 2007; Chung *et al*, 2009) which were conducted by the Agency for Healthcare Research and Quality (AHRQ). Prior to its considerations, the SACN vitamin D working group (WG) updated the evidence base to include studies published since the IOM report. It subsequently considered findings from studies identified in an AHRQ update of the evidence (Newberry *et al*, 2014).
12. While the WG considered the evidence on vitamin D relating to nutritional aspects, additional expertise on ultraviolet radiation exposure was provided by Dr John O'Hagan (PHE). Advice on adverse effects of high vitamin D intakes was provided by the Committee on Toxicity (COT). Information on the photobiology of vitamin D and on the effect of sunlight on endogenous vitamin D synthesis was provided by Professor Antony Young (King's College, London) and Professor Ann Webb (University of Manchester) respectively.

2. Biology and metabolism

13. Vitamin D plays an important role in the regulation of calcium and phosphorus metabolism and, therefore, in bone health (Jones, 1998).
14. Vitamin D is synthesised in the skin by the action of sunlight containing ultraviolet B (UVB) radiation. It can also be obtained from the diet. When skin is regularly exposed to sunlight, cutaneous production is, quantitatively, a more important source of vitamin D than diet (Holick *et al*, 1980). Dietary vitamin D supply becomes essential if there is insufficient cutaneous synthesis (generally caused by limited solar exposure during the summer and lack of UVB containing sunlight during the winter).
15. The two major forms of vitamin D are vitamin D₃ (also referred to as cholecalciferol) and vitamin D₂ (also referred to as ergocalciferol). In this report, the term vitamin D refers to both vitamin D₂ and D₃ unless the specific form is indicated.

Chemistry

16. Vitamin D is classified as a secosteroid. Vitamin D₂ (C₂₈H₄₄O) differs from vitamin D₃ (C₂₇H₄₄O) in the side chain attached to the secosteroid skeleton, which contains an additional methyl group on carbon atom 24 and a double bond between carbon atoms 22 and 23 (Norman, 2008) (see Figure 1). This difference means that the molecular mass of vitamin D₂ (396.65 g/mol) is 3.1% higher than that of vitamin D₃ (384.64 g/mol).



Units of measurement

17. Vitamin D intake is expressed in International Units (IU) or in micrograms (µg). IUs are based on antirachitic activity³ measured in bioassays using rats. One IU of vitamin D is defined by the World Health Organization (WHO) (1950) as the activity produced by 0.025 µg of crystalline vitamin D₃ (1 µg = 40 IU). Although this definition is based on vitamin D₃ activity, the conversion continues to be generalised to both forms of the vitamin regardless of the difference in their molecular mass.
18. Serum concentrations of circulating vitamin D and its metabolites are expressed as nanomoles per litre (nmol/L) or nanograms per millilitre (ng/ml); 2.5 nmol/L is equivalent to 1 ng/ml. However due to the differences in molecular mass, there is not absolute correspondence in the conversion of ng to nmol for vitamin D₂ and D₃ and their metabolites. The inconsistencies relating to the measurement of the two forms of vitamin D need to be considered in the interpretation of studies comparing vitamin D₂ and D₃.
19. Units of measurement used in this report are µg (intakes) and nmol/L (serum concentration); the corresponding amounts in IU (intakes) and ng/ml (serum concentration) are also provided in parentheses.

³ The amount required to prevent rickets.

Sources

20. Vitamin D is obtained by cutaneous production and from foods or dietary supplements containing either vitamin D₂ or D₃. Vitamin D₃ is the only form produced cutaneously. Vitamin D₂ is formed in plants by UVB exposure of the plant steroid, ergosterol.
21. A metabolite of vitamin D, 25-hydroxyvitamin D, is present in animal products.

Cutaneous synthesis

22. Vitamin D₃ is produced endogenously from 7-dehydrocholesterol (7DHC) in the skin of humans and animals by the action of sunlight containing UVB radiation (wavelength 280-315 nm) or by artificial UVB light. The 7DHC in the epidermis and dermis is converted to previtamin D₃, which reaches a maximum concentration in the skin within a few hours (Holick 1980). Even with prolonged irradiation in sunlight the amount of previtamin D formed is limited to 12-15% of the original 7DHC (McLaughlin *et al*, 1982; Webb *et al*, 1988). UVB intensity and skin pigmentation affect the rate of conversion but not the maximum amount produced in the skin (Holick *et al*, 1979; 1980; 1981).
23. Previtamin D₃ is thermodynamically unstable. It is converted to the more stable vitamin D₃ in an uncatalysed temperature dependent isomerisation reaction which takes place in the plasma membrane of epidermal and dermal cells over a period of 3 days (Holick *et al*, 1980). Prolonged UVB exposure results in conversion of previtamin D₃ to lumisterol and tachysterol which are biologically inactive (Holick *et al*, 1981). Cutaneous vitamin D₃ can also undergo photoconversion and isomerise into a variety of photoproducts including suprasterol I, suprasterol II and 5,6 transvitamin D₃ (Webb, 1989). These photoconversions, which are reversible if concentrations of previtamin D₃ fall, prevent accumulation of toxic amounts of vitamin D₃ from cutaneous exposure alone (Holick *et al*, 1980).
24. The amount of vitamin D₃ made in the skin depends on exposure of the skin to UVB radiation and efficiency of cutaneous synthesis (Webb, 2006; Holick, 2005). Exposure of skin to UVB radiation is affected by a number of factors such as time of day, season, latitude, altitude, cloud cover, air pollution, as well as clothing and sunscreen use. Efficiency of cutaneous vitamin D synthesis is decreased with darker skin pigmentation (Clemens *et al*, 1982) and increasing age (MacLaughlin & Holick, 1985).
25. Cutaneous synthesis of vitamin D, including factors affecting its production and adverse effects of sunlight exposure, is considered in more detail in section 2.

Dietary sources

26. In the UK, the main dietary sources of vitamin D are foods of animal origin, fortified foods and supplements. Commercial synthesis of vitamin D₃ and D₂ is by UVB irradiation of 7DHC (from sheep wool) and ergosterol (from plants and fungi) respectively (Bikle, 2009).
27. There are few naturally rich food sources of vitamin D. Foods that contain significant amounts are mostly of animal origin and contain vitamin D₃. Rich sources include egg yolk (~5 µg/100 g) and oily fish (5-10 µg/100 g) such as salmon, mackerel and sardines (FSA, 2002). Animal products such as muscle, fat, liver and kidney also contain vitamin D₃ (0.1-1.5 µg/100 g), as well as the vitamin D metabolite 25-hydroxyvitamin D (Ovesen *et al*, 2003).
28. Wild mushrooms are a rich natural source of vitamin D₂, containing approximately 13-30 µg/100g fresh weight (Mattila *et al*, 1994). Cultivated mushrooms do not contain high amounts of vitamin D₂ since they are grown in the dark.
29. Foods are fortified with either vitamin D₃ or D₂. In the UK, all margarine sold for domestic use was previously subject to mandatory fortification with vitamin D (and vitamin A) from 1940 until the

mandatory requirement was removed in 2013⁴. However, most margarines and fats spreads are presently still fortified with vitamin D on a voluntary basis. Other foods, such as breakfast cereals and dried or evaporated milks, may also be fortified on a voluntary basis.

30. European Union (EU) law (Directive 2006/141/EC) stipulates vitamin D fortification of infant formula (1-2.5 µg/100 kcal) and follow-on formula (1-3 µg/100 kcal).
31. In the US, almost all milk⁵ is fortified with vitamin D on a voluntary basis (9.6 µg/L; 385 IU/L) (Food and Drug Administration, 2009). Other foods fortified on a voluntary basis include breakfast cereals (about 75%), milk substitutes (slightly more than 50%), yoghurts (about 25%) and cheeses, juices, and spreads (8-14%) with amounts ranging from 1-2.5 µg (40-100 IU) per serving (Yetley, 2008). Addition of vitamin D to infant formula is mandatory⁶ (1-2.5 µg or 40-100 IU per 100 kcal).
32. In Canada, fortification of milk (0.8-1 µg or 33-45 IU per 100 ml) and margarine (13 µg or 530 IU per 100 g) with vitamin D is mandatory⁷ and fortified plant-based beverages (such as soy milk) must contain an amount equivalent to that in milk. Infant formula must also be fortified on a mandatory basis (1-2 µg or 40-80 IU per 100 kcal).
33. Dietary vitamin D supplements contain either vitamin D₂ or vitamin D₃. Vitamin D supplements can also administered by intramuscular injection. The contribution that supplements make to vitamin D intakes in the UK is considered in section 8.

Metabolism

Absorption of dietary vitamin D

34. Dietary vitamin D is lipid soluble and is absorbed with long-chain triglycerides in the small intestine (Haddad et al, 1993). Ingested vitamin D is incorporated into chylomicrons within the enterocytes and secreted through the lymph into the systemic circulation (Dueland *et al*, 1983).
35. Absorption of ingested vitamin D has been reported to range from 62 to 91% (Thompson et al, 1966). Intestinal malabsorption disorders may reduce vitamin D absorption due to a decreased ability to absorb lipids. A systematic review that evaluated the impact of different vehicles (powders, lipids, ethanol) on the absorption of vitamin D supplements reported that absorption was greatest in the oil-based vehicle (Grossman & Tangpricha, 2010); however, the authors noted the limited number of studies that have investigated this issue.
36. An RCT (Biancuzzo *et al*, 2010) that compared vitamin D absorption (25 µg/d) from fortified orange juice with that from vitamin D supplements over 11 weeks (n=86) reported no significant difference in serum 25(OH)D concentration between those consuming the fortified orange juice and those consuming supplements. However, this was a small study that did not specify whether the fortified orange juice was consumed with/without a meal. It is also possible that the vitamin D in the orange juice was enclosed in micelles, which would facilitate absorption.

Transport in the circulation

37. Dietary vitamin D₂ and D₃ are transported in chylomicrons via the lymph and blood plasma to the liver. Cutaneously produced vitamin D₃ enters the extracellular fluid before diffusing into dermal capillaries

⁴ Removed as part of the Government's *Red Tape Challenge* with the aim of reducing the 'overall burden of regulation' (<http://www.redtapechallenge.cabinetoffice.gov.uk/home/index/>)

⁵ Assumed that this refers to dairy fluid milk and not plant based beverages.

⁶ Food and Drug Administration . Code of Federal Register Title 21, Sec.107.100 infant formula: nutrient specifications. Available at <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRserach.cfm>

⁷ Food and Drug Regulation. Available online at http://laws.justice.gc.ca/PDF/Regulation/C/C.R.C._c._870.pdf

(Holick, 2011). After entering the circulation it is transported to the liver bound to vitamin D binding protein (DBP), which is synthesised in the liver (Haddad, 1995). Any vitamin D left in the chylomicron remnant is taken up by the liver for storage (Mawer *et al*, 1971)

38. The appearance of vitamin D in plasma is short-lived since it is either taken up by adipose and other tissues or metabolised in the liver (Mawer *et al*, 1972). The plasma half-life of the parent vitamin D is about 4-6 hours (Mawer, 1971).

Conversion to active metabolite

39. The active metabolite of vitamin D is 1,25-dihydroxyvitamin D (1,25(OH)₂D). Conversion of vitamin D to 1,25(OH)₂D occurs in two sequential hydroxylation steps (DeLuca, 1974) (see figure 2). The first is in the liver where vitamin D is hydroxylated to 25-hydroxyvitamin D [25(OH)D] which is the major circulating metabolite of vitamin D. The second hydroxylation step is in the kidney and other tissues where 25(OH)D is converted to 1,25(OH)₂D.
40. The difference in the side chain between vitamin D₂ and D₃ is maintained during metabolism, i.e., vitamin D₃ is converted to 25(OH)D₃ and then to 1,25(OH)₂D₃; vitamin D₂ is converted to 25(OH)D₂ and then to 1,25(OH)₂D₂ (Jones *et al*, 1998).
41. Although vitamin D₂ undergoes similar metabolic transformations to vitamin D₃, it is unclear if all details of regulation and biological activity are identical to those of vitamin D₃ (Henry, 2011). Vitamin D₂ and its metabolites have a lower binding affinity to DBP than vitamin D₃ and its metabolites (Houghton & Vieth, 2006).

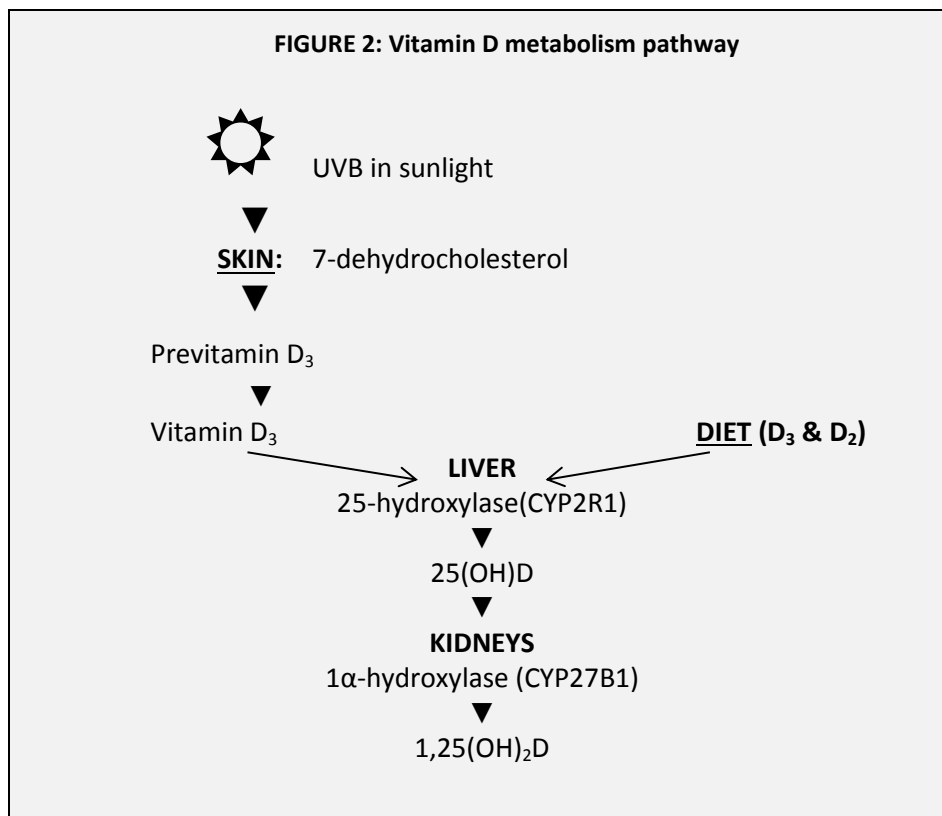
Hydroxylation to 25(OH)D

42. In the liver, hydroxylation of vitamin D to 25(OH)D is mediated by a cytochrome P450 (CYP) enzyme, identified as CYP2R1 (Henry, 2011). CYP2R1 appears to hydroxylate vitamin D₂ and D₃ with equal efficiency (Strushkevich *et al*, 2008). Other 25-hydroxylases have been found to have different activities for vitamin D₂ and vitamin D₃ (Bikle, 2009).
43. Following hydroxylation in the liver, 25(OH)D is secreted into the circulation where it binds to DBP and is transported to the kidney and some tissues for activation or breakdown. The 25(OH)D-DBP complex enters the kidney by receptor-mediated endocytosis, which is required to prevent loss of 25(OH)D in the urine. Two multi-ligand endocytic receptors, megalin and cubilin, which are strongly expressed in renal proximal tubules, are thought to be involved in the uptake of the DBP-25(OH)D complex by the kidney. The 25(OH)D-DBP complex can bind directly to megalin. The endocytic process is facilitated by cubilin which sequesters the complex on the cell surface before internalisation via megalin (Nykjaer *et al*, 2001). Megalin and cubilin are recycled back to the plasma membrane after intracellular release of the 25(OH)D-DBP complex.

Hydroxylation to 1,25(OH)₂D

44. In the kidney, 25(OH)D is further hydroxylated to 1,25(OH)₂D, its biologically active form, or to 24,25-dihydroxyvitamin D [24,25(OH)₂D]. Production of 24,25(OH)₂D is usually the first step in the metabolic pathway to inactivate 25(OH)D which prevents vitamin D intoxication (Norman, 2008). Serum concentration of 24,25(OH)₂D is directly related to 25(OH)D concentration.
45. The conversion of 25(OH)D to 1,25(OH)₂D is catalysed by CYP27B1, a mitochondrial P450 enzyme with 1 α -hydroxylase activity which is produced in the proximal renal tubule (Prentice *et al*, 2008; Bikle, 2009). Conversion to 24,25(OH)₂D is by the 24-hydroxylase enzyme, CYP24 (Norman, 2008).

46. CYP27B1 activity depends on the absolute intracellular concentration of 25(OH)D. The substrate concentration of 25(OH)D required for 50% maximal activity of the CYP27B1 enzyme is approximately 100 nmol (Henry, 2005). There is little correlation between serum concentrations of 25(OH)D and 1,25(OH)₂D (Lips, 2001; Vieth *et al*, 2003; Need *et al*, 2000).
47. The metabolic fate of 25(OH)D depends on calcium requirements. When calcium is required by the body, a greater proportion of 25(OH)D undergoes 1α-hydroxylation; a plentiful supply of calcium results in greater proportion of 24-hydroxylation (Jones, 1998).
48. Renal production, which is the principal source of 1,25(OH)₂D in the serum, mediates the functions of the vitamin D endocrine system. However, a number of other tissues also have the ability to produce 1,25(OH)₂D. CYP27B1 mRNA, CYP27B1 protein and enzyme activity have all been detected in at least nine extra renal tissues, including bone (van Driel *et al*, 2006; Kogawa *et al*, 2010; Zhou *et al*, 2010), skin (keratinocytes), placenta (decidua), breast, colon, prostate, endothelial cells, pancreatic islets and parathyroid glands (Norman, 2008) and macrophages (Crowle *et al*, 1987). Extra-renal 1,25(OH)₂D production does not generally increase 1,25(OH)₂D concentrations in the circulation (Norman, 2008) and its effects appear to be restricted to paracrine and autocrine functions within these tissues.



Physiological regulation

Regulation of 25(OH)D production

49. Serum 25(OH)D concentration is not subject to feedback regulation but appears to reflect vitamin D supply from cutaneous synthesis and the diet (Bhattacharyya & DeLuca, 1973). The half-life of serum 25(OH)D is about 2-3 weeks (Lund *et al*, 1980) while the half-life of 1,25(OH)₂D is about 4-6 hours (Holick, 2004; Brandi *et al*, 2002).

50. Serum 25(OH)D concentration is widely used as a biomarker of vitamin D status because it has a long half-life in the circulation and is not subject to tight homeostatic control (Norman, 2008; Bikle, 2009; DeLuca, 2008). Biomarkers of vitamin D status are considered in more detail in section 4.
51. Serum 25(OH)D concentration depends on the amount of vitamin D delivered to the liver, the amount produced by the liver and its half-life in serum (Prentice *et al*, 2008). These are affected by a number of factors including the amount of vitamin D entering the body, the amount of body fat and muscle mass, the rate of 25(OH)D uptake and rate of conversion to other metabolites (such as 1,25(OH)₂D, 24,25(OH)₂D). Other factors affecting serum 25(OH)D concentration include the volume of extracellular fluid and serum DBP concentration (Liang & Cooke, 2005; Dueland *et al*, 1983; Orwoll & Meier, 1986; Gascon-Barre, 2005; Lips, 2001; Bolland *et al*, 2007). Plasma concentration of 25(OH)D has been reported to decrease in response to acute inflammation (Reid *et al*, 2011). In addition, different polymorphisms of DBP have different affinities and transport efficiencies for 25(OH)D (Speeckaert, 2006). Evidence from children with calcium-responsive rickets (Pettifor, 1991) and from calcium-deficient rats (Clements *et al*, 1987) suggests that low calcium intakes may adversely affect vitamin D utilisation by increasing breakdown of 25(OH)D to inactive products that are excreted in the bile.

Regulation of 1,25(OH)₂D production

52. Synthesis of 1,25(OH)₂D in the kidney is tightly regulated. Upregulation is through the action of parathyroid hormone (PTH); down regulation is through fibroblast growth factor 23 (FGF23) and direct negative feedback by 1,25(OH)₂D itself (Henry, 2011).
53. Calcium-sensing proteins in the parathyroid gland stimulate PTH secretion in response to a fall in serum ionised calcium concentration. PTH stimulates production of the CYP27B1 enzyme in the proximal cells of the kidney (Bajwa, 2008) which increases renal synthesis of 1,25(OH)₂D (Henry, 2011). 1,25(OH)₂D exerts a direct negative feedback by down regulating the expression of the gene for CYP27B1, the enzyme required for its synthesis (Henry, 2011). It also exerts an indirect negative feedback by reducing secretion of PTH (Norman, 2008; Holick, 2011). Additionally, 1,25(OH)₂D induces its own degradation by stimulating production of the CYP24A1 enzyme, a 24-hydroxylase which converts 1,25(OH)₂D and 25(OH)D to water-soluble compounds which are excreted through bile (Jones, 1998).
54. FGF23 mediates the regulatory effect of serum phosphate concentrations on lowering 1,25(OH)₂D concentrations (Shimada *et al*, 2004). It is secreted by bone osteoblasts and osteocytes in response to increasing serum phosphate concentrations (Henry, 2011) and down regulates 1,25(OH)₂D synthesis by inhibiting renal transcription of CYP27B1 (Perwad *et al*, 2007). It also increases phosphate excretion in the urine by reducing the number of sodium-phosphate transporters in the renal brush border membranes (Segawa *et al*, 2007; Shimada *et al*, 2004).
55. Extrarenal CYP27B1 enzyme activity is not regulated by calcium and phosphate regulating hormones but may be affected by changes specific to the cell's environment or function (Henry, 2011).

Catabolism and Excretion

56. 24-hydroxylation is the first step in the inactivation of 25(OH)D and 1,25(OH)₂D (DeLuca, 2008). Degradation of both is catalysed by the 24-hydroxylase enzyme CYP24 (produced in the kidney) in a series of four successive reactions to produce inactive water-soluble compounds which are excreted in bile (Henry, 2011).
57. Through its inactivation of 1,25(OH)₂D, the CYP24 catalysed pathway plays an important role in limiting the hormone's effects in target tissues; 1,25(OH)₂D can increase the levels of CYP24 mRNA by two to three orders of magnitude above background amounts (Henry, 2011).

58. Studies in bird and mouse models suggest that 24,25(OH)₂D may also have a biological role in bone healing (Seo & Norman, 1997; St-Arnaud, 2010).

Storage/sequestration

59. Adipose tissue is considered to be the major storage/sequestration site for vitamin D (Mawer *et al*, 1972; Rosenstreich, 1971) although there is some evidence that muscle may also be a storage tissue for 25(OH)D (Girgis *et al*, 2014).
60. A number of studies have reported adiposity and body mass index to be inversely related to serum 25(OH)D concentrations (Parikh *et al*, 2004; Snijder *et al*, 2005; Arunabh *et al*, 2003; Liel *et al*, 1988) suggesting vitamin D is not readily available from adipose tissue and that, because of its lipophilic nature, it is sequestered rather than stored. This is supported by some studies which reported increases in serum 25(OH)D concentrations with weight reduction in obese individuals (Zitterman *et al*, 2009; Tzotzas *et al*, 2010).
61. Details about accumulation and mobilisation of vitamin D stores from adipose tissue and other tissues such as muscle are not clear at this time (IOM, 2011).

Mechanism of action

62. 1,25(OH)₂D elicits a biological response through the regulation of gene transcription (genomic response) and by activating signal transduction pathways at or near plasma membranes (non-genomic or rapid response) (Norman *et al*, 2004). The mechanism of action is mediated through binding with a single vitamin D receptor (VDR) (DeLuca, 2004). After formation in the kidney, 1,25(OH)₂D enters the circulation bound to DBP.
63. VDR has a high affinity for 1,25(OH)₂D₃ (Norman, 2008). 1,25(OH)₂D₂ and 1,25(OH)₂D₃ appear to be similar in their binding affinity to VDR (DeLuca, 2008). The VDR is expressed in cells involved in calcium and phosphate homeostasis, e.g., enterocytes, osteoblasts, parathyroid and distal renal tubule cells (Jones, 1998). VDRs are also present in a wide range of other cells and tissues not considered targets of vitamin D action, including macrophages, lymphocytes, skin keratinocytes, pancreatic β-islet cells, ovarian tissue, mammary epithelium, neuronal tissue, lung, gonads, prostate, placenta, and adipose tissue (Jones, 1998; Norman 2008).
64. The amount of VDR expressed in different tissues varies widely and appears to be regulated in some tissues (e.g., kidney, parathyroid) but not in others (Dame *et al*, 1986; Brown *et al*, 2007). Many VDR expressing cells also possess the enzyme CYP27B1 and therefore have the capacity to produce 1,25(OH)₂D (Bikle, 2009).

Genomic response

65. The VDR functions in the nucleus of cells as a heterodimer with a retinoid X receptor (RXR) to regulate vitamin D target genes. The heterodimeric complex interacts with vitamin D-responsive elements (VDREs), which are repeat sequences of 6 nucleotides separated by 3 nonspecified bases within the promoter region of target genes, resulting in activation or repression of transcription (Christokos *et al*, 2003; DeLuca *et al*, 2004; Rachez & Freedman, 2000).

Non-genomic response

66. Non-genomic responses of 1,25(OH)₂D are mediated by the interaction of the VDR with caveolae (membrane invaginations) which are present in the plasma membrane of a variety of cells (Huhtakangas *et al*, 2004). Upon activation by 1,25(OH)₂D, VDRs may elicit a cellular response on calcium channels through second messengers such as mitogen-activated protein kinase or cyclic adenosine monophosphate (Feldman

et al, 2005). Rapid response mechanisms, through membrane VDRs and second messengers operate in the intestine, vascular smooth muscle, pancreatic β -cells and monocytes (Lips, 2006).

Genetic influences on vitamin D metabolism

67. In addition to behavioural and environmental factors, twin and family studies suggest a genetic component to the inter-individual variability in serum 25(OH)D concentrations. Rates of heritability have been estimated to range from 29 to 80% (Shea *et al*, 2009; Hunter *et al* 2001).
68. Rare mutations in genes involved in vitamin D metabolism lead to functional vitamin D deficiency. For example, mutations in the genes coding for CYP27B1 and VDR cause vitamin D dependent rickets type I (VDDR I) (Fu *et al*, 1997) and vitamin D dependent rickets type II (VDDR II) (Malloy *et al*, 1999) respectively.
69. A number of more common polymorphisms⁸ in genes encoding proteins involved in vitamin D metabolism have been identified. Two meta-analyses of genome-wide association studies⁹ (Ahn *et al*, 2010; Wang *et al*, 2010) examined the influence of single nucleotide polymorphisms (SNPs¹⁰) in such genes on serum 25(OH)D concentrations. Ahn *et al* (2010) included 9 cohorts from the USA and Finland (discovery sample, n=4501; replication sample, n=2221). Genome-wide significant associations with serum 25(OH)D concentration were found for SNPs within genes encoding DBP (rs228769, rs7041, rs1155563), CYP2R1 (rs206079) and at the NADSYN1/DHCR7¹¹ locus (rs3829251). Wang *et al* (2010) included 15 cohorts from the USA, Canada and Europe (discovery sample, n=16,125; replication sample, n=17,744). SNPs at three loci reached genome-wide significance for an association with serum 25(OH)D concentration: rs2282679 in the DBP gene, rs12785878 near DHCR77 and rs10741657 near the CYP2R1 gene.
70. These findings suggest that common polymorphisms in genes involved in vitamin D metabolism might influence serum 25(OH)D concentrations. The functional relevance of these polymorphisms is not clear.

Biological activity of vitamin D₂ vs vitamin D₃

71. Vitamin D₂ and vitamin D₃ both elevate serum 25(OH)D concentration (Seamans & Cashman, 2009) and both have been shown to correct vitamin D deficiency rickets. However, there continues to be disagreement on whether they are equally effective in raising and maintaining serum 25(OH)D concentrations (Lanham-New *et al*, 2010; Cashman, 2011; Logan *et al*, 2013; Swanson *et al*, 2014). Several biologically plausible mechanisms have been suggested that could contribute to the greater capacity of vitamin D₃ over D₂ to maintain higher 25(OH)D concentrations over time (reviewed in Houghton & Vieth, 2006).
72. Results from studies that have compared their effectiveness in raising serum 25(OH)D concentration have been inconsistent. A meta-analysis of 7 studies (n=294) (Tripkovic *et al*, 2012) reported a significantly greater absolute increase from baseline in serum 25(OH)D concentration with vitamin D₃ (p=0.006); however, heterogeneity between the studies was high (I²=85%). Separate meta-analyses found a significantly larger increase in serum 25(OH)D concentration with vitamin D₃ compared with vitamin D₂ supplementation in 3 studies using single bolus doses (p=0.04) while no significant difference was found between vitamin D₂ and D₃ in 5 studies that used daily supplementation (p=0.06). Heterogeneity was much higher in the studies using a bolus dose (I²=87%) compared to those using daily supplementation (I²=44%).

⁸ Polymorphisms are genetic variants that occur at a frequency of at least 1% in the population.

⁹ The entire human genome is searched to identify associations with the phenotype of interest.

¹⁰ SNPs occur when a single nucleotide in the genome sequence is altered.

¹¹ NADSYN1 encodes nicotinamide adenine dinucleotide synthetase-1 which catalyses the final step in the biosynthesis of nicotinamide adenine dinucleotide. An SNP located in the DHCR7 gene, rs1790349, which is in high linkage disequilibrium with rs3829251, was also associated with serum 25OHd concentrations; because of the biological relevance of DHCR7 to vitamin D metabolism the authors refer to this as the NADSYN1/DHCR7 locus.

73. Conclusions regarding any differences in biological activity between vitamin D₂ and vitamin D₃ cannot be drawn from this meta-analysis because of a number of limitations, including: the small number and size of studies (n=19-89); variability in 25(OH)D assay methodology (see later); differences in dose size and frequency and in treatment and follow-up time. Additionally, the doses of vitamin D₂ and D₃ used in the studies were very high and effects may be different at lower doses.
74. Results from RCTs published after the meta-analysis by Tripkovic *et al* (2012) support the suggestion that vitamin D₃ is more effective than vitamin D₂ in raising serum 25(OH)D concentration (Logan *et al*, 2013; D₂-D₃ study). An RCT in New Zealand, conducted over winter, compared effects of 25 µg/d (1000 IU) of vitamin D₂, D₃ and placebo over 25 weeks on serum 25(OH)D concentration of adults (n=95; 18-50 y) (Logan *et al*, 2013). After 25 weeks, serum 25(OH)D concentrations of participants in the placebo group were significantly lower than those in the vitamin D₂ and D₃ groups. The mean serum 25(OH)D concentration was significantly lower in the vitamin D₂ supplemented group compared with the vitamin D₃ supplemented group (p<0.001).
75. In the largest vitamin D₂ vs vitamin D₃ RCT conducted (Lanham-New, personal communication, 2015¹²), vitamin D₃ fortification at a dose of 15 µg/d (600 IU) over 12 weeks during the winter months (November to March) was shown to be much more effective (p<0.001) in raising serum 25(OH)D concentration than vitamin D₂ in white (n=245) and South Asian (n=90) women (mean age, 43.7±12.8 y) living in Southern England. The food/drink matrices of a biscuit/juice did not affect the results and both forms of vitamin D were bioavailable in these solid and liquid foods. Serum 25(OH)D concentrations decreased in both placebo groups.

Toxicity

76. Vitamin D toxicity can lead to hypercalcaemia, which results in deposition of calcium in soft tissues, diffuse demineralisation of bones and irreversible renal and cardiovascular toxicity. Hypercalcaemia can also lead to hypercalcuria (EVM¹³, 2003).
77. Prolonged sunlight exposure does not lead to excess production of cutaneous vitamin D because endogenously produced pre-vitamin D₃ and vitamin D₃ is photolysed to inert compounds (see paragraph 23). High doses of oral vitamin D supplements have, however, been shown to have toxic effects (Vieth, 2006). Cases of vitamin D toxicity resulting from ingestion of over-fortified milk have also been reported (Jacobus *et al*, 1992; Blank *et al*, 1995).
78. Animal studies suggest that serum 25(OH)D concentrations associated with toxicity are above 375 nmol/L (Jones, 2008). Evidence on vitamin D toxicity in humans is based on anecdotal case reports of acute accidental vitamin D₂ or D₃ intoxication resulting in 25(OH)D concentrations of 710-1587 nmol/L and a threshold for toxic symptoms at concentrations of about 750 nmol/L (Vieth, 1990). Hypercalcaemia has been reported at plasma concentration above 375-500 nmol/L (Vieth, 1990; Jones, 2008).
79. The mechanism of how vitamin D toxicity might arise is presently unclear. Proposed mechanisms are based on increased concentrations of the active metabolite of vitamin D reaching the VDR in the nucleus of target cells and causing gene over expression. Three main hypotheses have been proposed (Jones, 2008): plasma concentrations of 1,25(OH)₂D are increased leading to increased cellular concentrations of 1,25(OH)₂D; plasma 25(OH)D concentrations exceed DBP binding capacity and free 25(OH)D enters the cell and has direct effects on gene expression; or, concentrations of a number of vitamin D metabolites, especially vitamin D itself and 25(OH)D, exceed the DBP binding capacity causing release of free 1,25(OH)₂D which enters target cells.

¹² The D2-D3 Study; BBSRC DRINC Grant no. BB/I006192/1.

¹³ Expert Group on Vitamins and Minerals.

80. Effects of high intakes of vitamin D, including thresholds for risk, single-dose acute toxicity vs sustained exposure, are considered in more detail in section 7.

Physiological role

Calcium & phosphate regulation

81. The major function of $1,25(\text{OH})_2\text{D}$ is regulation of calcium and phosphorus metabolism which is essential for bone mineralisation (DeLuca, 2008). Calcium homeostasis is also important for neuromuscular function (Holick, 2011).
82. Serum calcium concentration is tightly regulated and maintained at approximately 1 mmol/L ionised calcium or 2.5 mmol/L of total calcium (Rasmussen & DeLuca, 1963). A slight decrease is detected by calcium-sensing transmembrane proteins in the parathyroid gland inducing secretion of PTH into the circulation within seconds (Silver *et al*, 1996). PTH induces osteoblasts and proximal convoluted tubule cells in the kidney to produce $1,25(\text{OH})_2\text{D}$ which increases serum calcium concentration by stimulating intestinal calcium absorption, renal calcium reabsorption and bone resorption. The subsequent increase in serum calcium concentration is sensed by the parathyroid glands and PTH secretion is decreased (DeLuca, 2004).
83. In the intestine, $1,25(\text{OH})_2\text{D}$ interacts with the VDR to enhance expression of an epithelial calcium channel and a calcium binding protein (calbindin 9k) which increases calcium transport from the intestinal lumen into the circulation (Christakos *et al*, 2003). In the skeleton, $1,25(\text{OH})_2\text{D}$ interacts with VDR in the osteoblast to increase expression of receptor activator of NF κ B ligand (RANKL); this increases the production of osteoclasts which release calcium into the circulation (Christakos *et al*, 2003).
84. If serum calcium concentration exceeds the normal physiological range then the thyroid gland secretes the peptide calcitonin, which blocks calcium mobilisation from bone to restore homeostasis (Chambers & Magnus, 1982).
85. $1,25(\text{OH})_2\text{D}$ also increases phosphate absorption in the small intestine and induces secretion of FGF23 by osteocytes, increasing phosphate excretion in the kidney and thus preventing phosphate accumulation in the body (Kolek *et al*, 2005; Liu *et al*, 2008).

Physiological requirements by life-stage

Infants

86. Vitamin D, together with calcium and phosphorus, is required during infancy and early childhood (< 3 years) to meet the demands of rapid growth for healthy skeletal development. Prolonged deficiency of vitamin D during periods of bone growth in children leads to an accumulation of excess unmineralised osteoid (bone matrix); the low mineral to bone matrix ratio results in rickets. The main signs of rickets are skeletal deformity with bone pain or tenderness; and muscle weakness. Deficiencies of calcium and phosphorus can also cause rickets.

Children and Adolescents

87. Vitamin D is important for bone accretion during this time of skeletal development. Adolescence is a critical developmental period for bone health when there is rapid growth. Although rickets is most commonly observed during infancy it can also occur during the pubertal growth spurt and adolescence. Insufficient vitamin D during this time could also affect bone mineral density and lead to children and adolescents not achieving their full potential at peak bone mass.

Adults

88. In adults, vitamin D is required to maintain healthy bone. Deficiency can lead to osteomalacia, presenting as muscle weakness and bone tenderness or pain in the spine, shoulder, ribs or pelvis (DH 1991, 1998).
89. In addition to evidence suggesting a link between vitamin D status and rickets/osteomalacia, numerous epidemiological studies have reported associations between vitamin D status and other health outcomes including osteoporosis, tuberculosis, diabetes mellitus, multiple sclerosis, preeclampsia and cancer. The association between vitamin D status and health outcomes is considered in subsequent sections.

Pregnancy and lactation

90. The role of vitamin D during pregnancy and in the formation of the fetal skeleton is not clear. Vitamin D supplements (10 µg/400 IU/d) are currently recommended during pregnancy so that the fetus is not deficient in vitamin D and to avoid neonatal hypovitaminosis (DH 1991, 1998).
91. Breast milk is considered not to be a significant source of vitamin D or its metabolites. The vitamin D content of breast milk is variable across different studies because it depends on the type of milk measured (hind or fore) and the time of day it is collected. For some breast fed babies, vitamin D contained in breast milk could make a significant contribution to their vitamin D intake. It is also possible that babies utilise vitamin D more efficiently than adults.

3. Photobiology of vitamin D

Ultraviolet radiation

92. The sun is the main source of ultraviolet radiation (UVR) for most of the population. Artificial sources of UVR may provide a significant proportion of the exposure for specific groups including those who use artificial tanning facilities and those receiving UVR medical treatments.
93. Solar UVR forms the part of the electromagnetic spectrum from wavelengths of about 100-400 nm. The International Commission on Illumination (CIE¹⁴, 2011) has defined sub-regions of the UVR spectrum which take account of the transmission of the UVR in human tissue and potential health effects, into the following categories: UVA (315 – 400 nm); UVB (280 – 315 nm); UVC (100 – 280 nm).
94. The broad spectrum and intensity of the UVR emitted from the sun are due to its high surface temperature. The quantity and spectral distribution of solar radiation at the Earth's surface depend on the power output of the sun, the path of the radiation through the Earth's atmosphere, and the transmission properties of the atmosphere. Solar UVR undergoes absorption and scattering as it passes first through the outer layers of the atmosphere and then the stratosphere and the troposphere before reaching the Earth's surface. The most important of these processes are absorption by molecular oxygen and absorption by ozone.
95. The stratospheric ozone layer, formed between 10 and 40 km above the Earth's surface, prevents almost all UVR of wavelengths less than 290 nm (UVC) and a substantial proportion (70%–90%) of UVB radiation from reaching the Earth. Therefore, the ground level component of the solar UVR spectrum consists of wavelengths in the range of about 290 to 400 nm. This means that only UVA and UVB are relevant to human health. UVB accounts for about 5% of terrestrial UVR, the remainder being UVA.
96. Ground-level UVR consists of two major components, namely radiation received directly from the sun and radiation that has been scattered by the atmosphere. The ratio of the scattered to direct radiation varies with wavelength and with solar zenith angle (at 0° the sun is directly overhead and at 90° is on the horizon from a horizontal viewpoint). The ratio increases as the wavelength decreases and the solar zenith angle increases: UVB is scattered more than UVA and the amount of scattering increases as the sun moves from above towards the horizon.
97. Human exposure to solar UVR depends on the amount of sunlight available (climate), then the time spent outdoors and the level of exposure. The amount of sunlight available is determined primarily by solar elevation (which depends on time of year and time of day) and weather (which will influence outdoor activity and skin exposure). At middle-high latitudes where there are distinct seasons, the winter months are characterised by low solar elevation, short day length and cloudy skies which all reduce the available solar UVR.
98. The solar zenith angle depends on season, time of day and latitude (Webb, 2006). When the solar zenith angle is small (in summer, noon, at low latitudes) the sun is high in the sky and UVR has a relatively short path through the atmosphere. When the solar zenith angle is increased (in the early morning, late afternoon and during winter, high latitudes), UVR has to pass through more ozone which means that less UVB reaches the Earth's surface. In the UK, the spectral UVR irradiance (wavelength 300 nm) is theoretically at a maximum at solar noon (GMT), when the solar zenith angle is at its lowest. This is at least about ten times higher than that over the period before 09:00 GMT or after 15:00 GMT. Seventy per cent of the global UVR exposure¹⁵ is delivered during the four hours centred around noon.

¹⁴ From its French title: Commission Internationale de l'Éclairage

¹⁵ The integrated total exposure dose of biologically weighted UVR falling on a horizontal surface.

Biological effects of solar UVR exposure

99. Solar UVR has been associated with beneficial and harmful biological effects. Synthesis of vitamin D is the only established benefit of solar UV exposure. Exposure of skin to solar UVR in the UVB spectral region initiates synthesis of vitamin D. Skin epidermal 7-DHC located in the cell membranes is converted to pre-vitamin D, which isomerises into vitamin D over a period of 12-24 hours. The amount of UVB in a given solar spectrum depends on the height of the sun, which is a function of latitude, season and time of day (see Figures 3a and 3b).
100. Adverse biological effects of UVR exposure include damage to the skin (erythema or sunburn, photoageing and skin cancer) and eyes (photokeratitis, cataract and age-related macular degeneration). Public health advice focusses on sun avoidance and protection to reduce the risk of sunburn and skin cancer.

Erythema and skin cancer

101. Two measures are used to quantify erythema risk: the standard erythemal dose (SED) and the minimal erythemal dose (MED). SED is a fixed physical quantity, equal to 100 J/m^2 . The MED varies for each individual because the amount of UVR required to produce a just-measurable degree of erythema (sunburn or redness) depends on skin type, time of year, behaviour and possibly age; one MED is the minimum dose of UVR that produces erythema in that person's skin. The Fitzpatrick scale is the most commonly used numerical skin classification scheme for human skin colour (Fitzpatrick, 1975; 1988). Skin type is classified into 6 categories according to its response to UVR, from most sensitive (type I/fair skin) to least sensitive (type VI/dark brown or black skin).
102. One of the main challenges in establishing the link between UVR exposure and adverse health effects is determining personal dosimetry, whether from solar or artificial sources. Whilst it is relatively straightforward to determine ambient levels of UVR, the actual skin exposure of any one person is difficult to assess.
103. The biological effects of UVR vary with wavelength; the variation of a given effectiveness function with wavelength is referred to as the action spectrum for that effect. Biological efficacy, which is more important than the relative amounts of UVB and UVA in sunlight, is determined by weighting solar UVR spectra with the action spectrum (i.e., wavelength dependence) of a given photobiological outcome such as erythema or vitamin D synthesis.
104. The action spectrum for erythema, in which UVB is orders of magnitude more effective than UVA per unit physical dose (J/cm^2), is very well established. Figures 4a and 4b show the effects of weighting Figs 1a and 1b with the CIE erythema action spectrum. Thus, the minority UVB physical component becomes the major biological component.
105. Skin cancer is initiated by UVR-induced damage to epidermal DNA. The most important photolesion is the cyclobutane pyrimidine dimer (CPD). The action spectrum for CPD formation is very similar to that for erythema and CPD is thought to be a trigger for erythema. The presence of erythema indicates that the skin has been over exposed to the sun and an association has been found between sunburnt skin and markers of DNA damage. Erythema may, therefore, be seen as a clinical surrogate for DNA photodamage that has carcinogenic potential. For an individual who does not burn but has regular sunlight exposure, there is some evidence that lifetime cumulative skin exposure to UVR is a risk factor for non-melanoma skin cancers such as squamous cell carcinomas.
106. There is considerable overlap in the UVB region between the action spectra for the formation of pre-vitamin D and erythema (and therefore DNA damage) as shown in Figure 5. This means that avoiding the

sun, especially around noon, to reduce the risk of sunburn (and CPD formation and skin cancer¹⁶) is likely to also reduce vitamin D synthesis.

CIE spectrum for photoconversion of 7DHC to pre-vitamin D

107. The validity of the official CIE action spectrum for the photoconversion of 7DHC to pre-vitamin D (see figure 5) has been disputed. However, it continues to be used to calculate the vitamin D efficacy of solar UVB under different climatic conditions and these calculations have been used for risk/benefit assessments. For example, one study suggests that the best time to obtain vitamin D is around noon because the relative efficacy for vitamin D production is greater than that for erythema (Sayre & Dowdy, 2007). The risk benefit calculations were based on the pre-vitamin D action spectrum and assumed that the action spectrum is accurate and that there is no spectral interaction. There is uncertainty about both these assumptions.
108. One study using *ex vivo* neonatal foreskin has suggested that UVA degrades vitamin D (Webb *et al*, 1989) but this has not been investigated *in vivo*.

Effect of latitude on dermal synthesis of vitamin D

109. The extent of the effect of latitude on vitamin D synthesis in the UK is not clear. While it probably has some effect, it could be relatively small compared to other factors. A study which compared serum 25(OH)D concentrations in postmenopausal women in Aberdeen (northern) and Guildford (southern) reported a difference of approximately 10 nmol/L (ref); however, the difference might not be due to solar radiation. Although UVB (as a proportion of UVR) lessens with increasingly northern latitudes, the weather also gets progressively colder which means that people go outdoors less; there are also differences in the area of skin exposed.

Seasonal variation in serum 25(OH)D concentration

110. There is a well-observed seasonal cycle in serum 25(OH)D concentrations in the UK and populations at mid-high latitudes (Poskitt *et al*, 1979; Beadle *et al*, 1980; Devgun *et al*, 1981; Livesey *et al*, 2007) which relates to the greater UVB content of solar UVR in summer.
111. During winter, the small amount of UVB in sunlight is insufficient to initiate synthesis of any biologically relevant quantities of vitamin D (Webb *et al*, 1988; Webb *et al*, 1989). In the UK, sunlight induced vitamin D synthesis in the white-skinned populations becomes effective from March with maximum serum 25(OH)D concentrations observed in September after a summer of exposure followed by a decline from October onwards through the winter months until the following spring. Webb *et al* (2010) measured serum 25(OH)D concentrations of white adults (n=125; age, 20-60 y) every month over 1 year and reported that the mean concentration was highest in September (71 nmol/L) and lowest in February (46 nmol/L).
112. A seasonal difference in serum/plasma 25(OH)D concentration of the UK population has also been observed in national surveys (see section 8).
113. The metabolic implications of seasonal variation in serum 25(OH)D concentration is currently unknown.

Effect of skin pigmentation on dermal synthesis

114. Epidemiological studies consistently show that, under given climatic conditions, people with darker skin colour have lower serum 25(OH)D concentrations than those with lighter skin colour (Harris & Dawson-Hughes, 1998; Hannan *et al*, 2008). It is not clear if this is due to different requirements or different lifestyles (e.g., sun avoidance behaviour). However, darker skin is only one of many factors, including

¹⁶ Non melanoma skin cancer has an action spectrum that is similar to erythema.

cultural (e.g., wearing concealing clothing) and biological (e.g., genetic background), that might affect the serum 25(OH)D concentration of different ethnic groups.

115. The pigment melanin, which gives skin its brown or black colour, absorbs UV radiation (Clemens *et al*, 1982). People with naturally brown or black skin are therefore less susceptible to sunburn and skin cancer than those with white skin. Skin pigmentation also reduces vitamin D synthesis from sunlight exposure by absorbing some of the incident radiation that would otherwise be absorbed by 7-DHC (Holick *et al*, 1981). If the absolute dose of UVB radiation is the same as that given to a person with white skin then people with darker skin will synthesise less vitamin D; however, darker skin has the same capacity to synthesise vitamin D if the dose of radiation is adjusted for the protective effect of melanin (Farrar *et al*, 2013).
116. Results from laboratory studies that have examined the role of melanin have been contradictory. A study which included participants with various skin tones (n=72), who had 90% of their skin exposed to UVB light (20-80 mJ/cm²) 3 times a week for 4 weeks, reported that 80% of the variation in treatment response was explained by UVB dose and skin tone (Armas *et al*, 2007). Farrar *et al* (2011) examined the effect of a controlled dose of UVR exposure (3x/week for 6 weeks) in individuals of South Asian ethnicity (n=15; aged 20-60 y; skin type V) exposing about 35% skin surface area. The study was conducted in January-February when ambient UVB is negligible in the UK to avoid confounding by lifestyle factors. Effects were compared with those of white-skinned individuals (n=109; age, 20-60y) who had been treated with the identical UV-radiation exposure in a previous study (Rhodes *et al*, 2010). The mean increase in serum 25(OH)D concentration was 11 nmol/L in South Asian individuals compared with 26 nmol/L in white-skinned individuals (p<0.0001).
117. Another study (Bogh *et al*, 2010) found no significant correlations with constitutive or facultative skin pigmentation and serum 25(OH)D concentration. In addition, no differences in the increase in serum 25(OH)D concentration was found between light and dark skinned groups after identical UVB exposure. However, this was a small study (9 pairs with a range of skin types in each group) which used phototherapy UVB sources containing non-solar UVB radiation (< 295 nm). These wavelengths penetrate the skin less well and pre-vitamin synthesis may occur above the melanin layer.

Effect of aging on dermal synthesis

118. Lower serum 25(OH)D concentrations have been reported in older people (Nordin *et al*, 1980). The amount of 7-DHC in skin decreases with increasing age (MacLaughlin & Holick, 1985). It has been inferred from this that the ability to synthesise vitamin D also decreases with age and suggested that this could explain lower serum 25(OH)D concentrations observed in older people (Lester *et al*, 1997). However it is uncertain whether the lower 7DHC concentration is a limiting factor if there is ample exposure to sunlight.
119. It has also been suggested that the lower concentrations of serum 25(OH)D reported in older people is a consequence of wearing more clothes and spending less time outdoors but this observation is based on earlier cross-sectional studies in the UK of older people who were not very active (Corless *et al*, 1975; Lester *et al*, 1977). These assumptions may no longer be valid since people are living longer and many older people remain active. A study in Boston (USA) that compared nursing home residents (with/without 10 µg/d vitamin D supplements) and free-living older people, reported that the supplements kept year round serum 25(OH)D concentrations > 37.5 nmol/L. This was similar to serum 25(OH)D concentrations of the free-living older people in spring and summer.
120. It is possible that lower serum 25(OH)D concentrations in older people could also be due to the development of conditions that become more common in old age (such as reduced liver and/or kidney function) but lower serum 25(OH)D concentrations are more generally due to reduced exposure to

sunlight rather than impaired metabolism of vitamin D. However, severe liver disease may impair hydroxylation of vitamin D to 25(OH)D.

121. The UK National Diet and Nutrition Survey (2008/9-2011/12) did not show a lower mean plasma 25(OH)D concentration in adults aged over 65 y compared to those aged 19-64 y; there was also no difference in the proportion with plasma serum 25(OH)D concentrations < 25 nmol/L.

Effect of sunscreen on dermal synthesis

122. Sunscreen use is recommended for the prevention of sunburn and skin cancer, which has raised concerns that sunscreen use may inhibit or prevent vitamin D synthesis. It is not possible to draw definitive conclusions from published studies (Springbett, Buglass & Young, 2010). An intervention study (Young, personal communication) compared the effect of proper use of sun protection factor (SPF) sunscreen with discretionary use during one week in Tenerife in March after winter in Poland. The intervention group was provided with sunscreen (SPF=18) and instructed in its correct use to avoid sunburn. The non-intervention group used their own products and were not given any instructions. Serum 25(OH)D concentration increased significantly in both groups, but the increase in the non-intervention group (~30 nmol/L) was about twice that of the intervention group (~15 nmol/L). Overall, the data suggest that vitamin D synthesis is still possible when sunscreens are used at the application density used for SPF testing.

Comparison of sunlight exposure and vitamin D supplementation as determinants of serum 25(OH)D concentration

123. Few studies have compared the effect of UVR with vitamin D supplementation on serum 25(OH)D concentration. One randomised trial compared the effect of full body narrow band solar range UVB (311 nm) (3x week) for 6 weeks with a daily dose of vitamin D₃ (40 µg/1600 IU) in participants (n=73) with serum 25(OH)D concentrations ≤ 25 nmol/L (Bogh *et al*, 2012). A greater increase in mean serum 25(OH)D concentration was found in the UVB treated group (from 19.2 to 75.0 nmol/L) compared with the vitamin D₃ supplemented group (from 23.3 to 60.6 nmol/L) (p=0.02).
124. Similar findings were reported in a 4-week study (Ala-Houhala *et al*, 2012) in which participants (n=63) with serum 25(OH)D concentration < 75 nmol/L were randomised to receive narrow band solar radiation exposures (3x week) with vitamin D₃ supplements (20 µg/d; 800 IU). Mean (SD) baseline serum 25(OH)D concentration of participants was 53 (±10.4) nmol/L. Narrow band UVB was more effective than supplements, with increases of 41.0 and 20.2 nmol/L respectively. The difference between the two treatments was significant at 2 weeks (p = 0.033) and 4 weeks (p < 0.001).

Current recommendations regarding sun exposure

125. NHS Choices advice on safe sun exposure¹⁷ follows that from Cancer Research UK's Sunsmart¹⁸:
- Spend time in the shade between 11am and 3pm.
 - **M**ake sure you never burn.
 - **A**im to cover up with a T-shirt, hat and sunglasses.
 - **R**emember to take extra care with children.
 - **T**hen use factor 15+ sunscreen.
126. The British Photodermatological Group (British Association of Dermatology) is similar to the above (i.e., avoid sunlight exposure between about 11am and 3 pm or seek shade and wear appropriately protective clothing if sunlight exposure between these times is unavoidable). However, it advises liberal use of SPF sunscreen of SPF 30 or more shortly before exposure and then again every couple of hours or so (and after

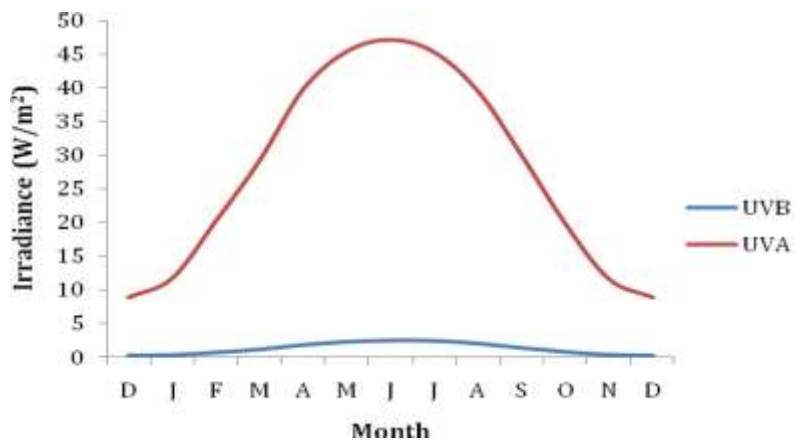
¹⁷ <http://www.nhs.uk/Livewell/skin/Pages/SunSAFE.aspx>

¹⁸ <http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/sun-uv-and-cancer/ways-to-enjoy-the-sun-safely>

swimming or exercise). It warns that failure to apply sunscreen correctly will result in much reduced protection (often less than a third of the protection stated) and that sunscreens should not be used to stay outside longer or to avoid more reliable protective measures such as clothing and shade.

FIGURE 3 - Variation of UVB and UVA irradiance at Chilton, UK (a) monthly at noon on the 21st day of each month (b) daily at summer and winter solstices. These data have been modeled taking total ozone into account.

(a)



127.

(b)

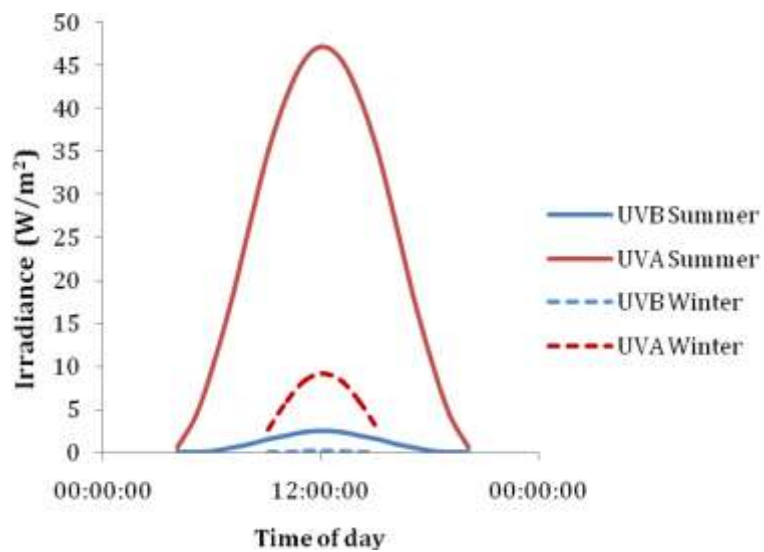
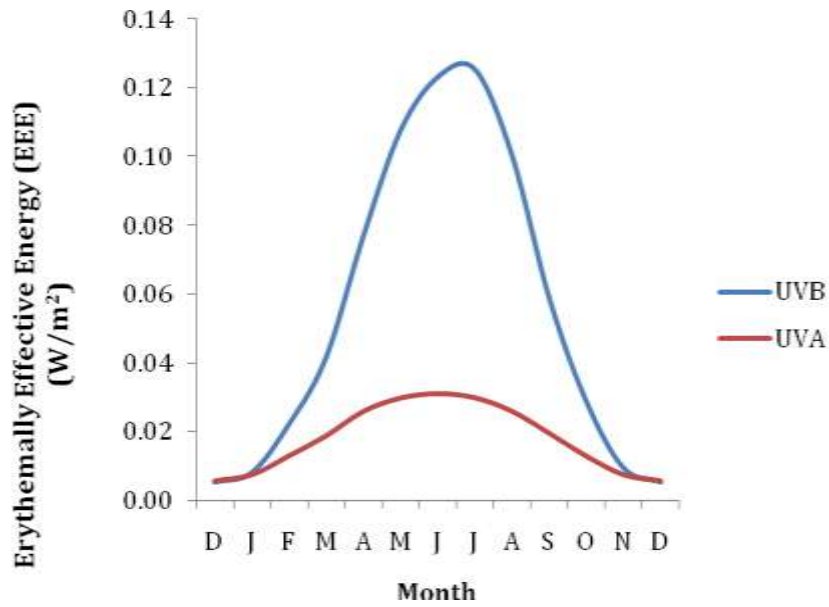


FIGURE 4 - Variation of UVB and UVA erythemally effective energy at Chilton, UK (a) monthly at noon on the 21st day of each month (b) daily at summer and winter solstices (data are based on the data in Fig 1)

(a)



129.

(b)

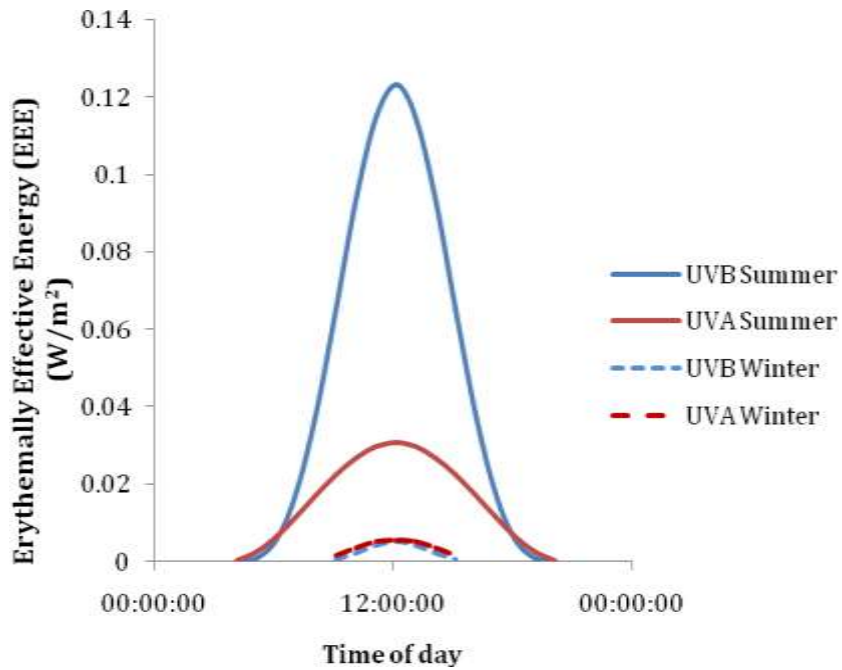
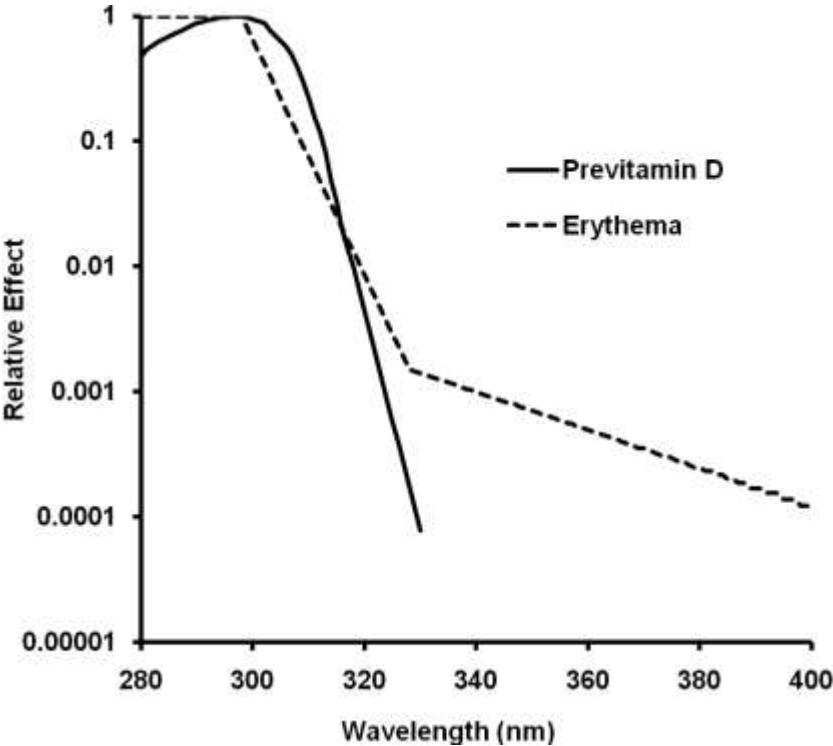


FIGURE 5 - CIE action spectra for erythema and the formation of pre-vitamin D. Note considerable overlap in the solar UVB region



4. Measuring vitamin D exposure (from diet and skin synthesis)

Biochemical markers of vitamin D exposure

127. Vitamin D status should reflect the body content of the vitamin and the amount available for cellular use. Indices of vitamin D status should allow determination of whether an individual has replete or depleted vitamin D body content.
128. It has been suggested that vitamin D can be stored in body fat (adipose tissue) due to the hydrophobic nature of adipose tissue. However, the extent to which the processes of accumulation and mobilisation are regulated by normal physiological mechanisms remains unknown at present (IOM, 2011). It may be a similar situation for skeletal muscle, but even less is known about this. This is important because vitamin D taken up by peripheral tissues that express lipoprotein lipase, especially adipose tissue and skeletal muscle, may reduce the amount in the circulation that can be presented to the liver for 25-hydroxylation. A greater understanding of the influence of body weight and body composition on the response of serum 25(OH)D concentration to vitamin D intake/exposure has been highlighted as an information gap and research need (IOM, 2011; Cashman & Kiely, 2011). In addition, once vitamin D enters the circulation from the skin or from the lymph, it is cleared by the liver within a few hours. Since it is not feasible to easily measure vitamin D and 25(OH)D concentrations in adipose or muscle tissue, or in the liver, the reliance is on biochemical assessment of 25(OH)D in a blood sample.
129. In the literature, vitamin D status refers only to the concentration of 25(OH)D in the serum. It does not include vitamin D or its metabolites in fat or elsewhere, which might be quickly mobilised. This means that rather than examining the relationship between vitamin D exposure (from diet and skin synthesis) and status, what is actually being considered in the literature is the relationship between exposure and serum 25(OH)D concentration.
130. The appearance in serum/plasma of the parent compound, vitamin D, is short-lived since it is either taken up by adipose tissues (and possibly muscle) or metabolised in the liver (Mawer *et al*, 1972). Heaney *et al* (2008) reported rapid and near-quantitative conversion of vitamin D₃ to 25(OH)D concentration at typical inputs of vitamin D₃ (whether cutaneous or oral). They also suggest that serum 25(OH)D concentration serves not only as a status indicator of the nutrient but as the principal storage form in the body. The 25(OH)D in circulation might be viewed as a storage form in the context that it is accessible to cells for utilisation, either directly in those cell types which possess a functional 1 α -hydroxylase enzyme, or indirectly following renal conversion of 25(OH)D to 1,25(OH)₂D. While circulating parent vitamin D can be measured using extensive HPLC analysis, this is not routinely performed, and not used clinically (Norman, 2008).
131. Although 1,25(OH)₂D is the key driver of physiological responses to vitamin D, there are a number of important reasons why it does not reflect exposure to vitamin D (Holick, 2004; SACN, 2007; IOM, 2011): plasma concentration of 1,25(OH)₂D is homeostatically regulated; concentrations are not directly regulated by vitamin D intake but by other factors (such as serum PTH); even in the presence of severe vitamin D deficiency, 1,25(OH)₂D concentration may be normal or even elevated as a result of up-regulation of the CYP27B1 enzyme; serum 25(OH)D concentration is about a thousand-fold higher than 1,25(OH)₂D concentration and its half-life is about 2-3 weeks compared to that of serum 1,25(OH)₂D, which is less than 4 hours.
132. There is consensus that serum/plasma 25(OH)D concentration should be used to assess vitamin D status because it reflects the contributions from both diet and dermal synthesis. Serum 25(OH)D concentration has been shown to reach an equilibrium after 6-8 weeks of vitamin D supplementation in adults (18-85y) (Harris & Dawson-Hughes, 2002; Viljakainen *et al*, 2006). A systematic review of existing and potentially

novel functional markers of vitamin D status reported that serum 25(OH)D concentration was increased in response to supplemental vitamin D intake in all the included RCTs irrespective of whether vitamin D₂ or D₃ was used, differing analytical techniques, study duration (6 weeks to > 2 years), or age group of participants (Seamans & Cashman, 2009).

133. Serum/plasma 25(OH)D concentration was used as an indicator of vitamin D status by the IOM Dietary Reference Intakes (DRI) committee on calcium and vitamin D in North America (IOM, 2011) and the UK and EU authorities (Department of Health, 1991, 1998; Scientific Committee for Food, 1993; German Nutrition Society, 2012; Health Council of the Netherlands, 2012; Nordic Council of Ministers (NORDEN), 2013) to establish dietary reference intake/values for vitamin D. However, the extent to which serum 25(OH)D concentration serves as a biomarker of effect is not clearly established; i.e., whether serum 25(OH)D concentrations relate to health outcomes via a causal pathway and can serve as predictors of such health outcomes (IOM, 2011).
134. A clearer understanding of the limitations of serum 25(OH)D concentration as a marker of exposure and status will provide for a better understanding of its relationship to specific health outcomes. For example, a study of patients who underwent elective knee arthroplasty has raised concerns about the reliability of serum 25(OH)D concentration as status marker in the face of significant systemic inflammatory insult (Reid *et al*, 2011). By day 2 post-operatively there was a large increase in C-reactive protein (CRP)¹⁹ concentrations and a significant decrease in plasma 25(OH)D concentration of ~40%. CRP, 25(OH)D, and calculated free 25(OH)D (i.e., 25(OH)D not associated with DBP or albumin) had not returned to pre-operative concentrations by 5 days post-operatively and, even at 3 months, 25(OH)D and free 25(OH)D concentrations remained significantly lower (20% and 30%). Mechanisms for the decrease in plasma 25(OH)D concentration were not evident.
135. Other lipid-soluble vitamins (A, E, K and some carotenoids) decrease during the systemic inflammatory response. While changes in CRP in chronic inflammatory conditions are likely to be of a lesser magnitude than those seen after knee arthroplasty, low serum 25(OH)D concentration has been associated with many of these diseases. The study by Reid *et al* (2011) therefore raises an important question in relation to reverse causality: low serum 25(OH)D concentration may be a consequence of diseases with an inflammatory component and not the cause.
136. It has also been suggested that the value of serum 25(OH)D concentration as an indicator of vitamin D exposure and status is limited by a number of other factors including its role as a pro-hormone rather than as a nutrient *per se* and its variability due to a number of non-nutritional factors which include: season, geographic latitude, clothing, institutionalization, use of sunscreen as well as physiological state of the individual such as body mass index, extracellular volume, and DBP concentration and affinity, variation between individuals in the half-life of 25(OH)D, and the effect of genetic variation (Brannon *et al*, 2008; IOM, 2011; Cashman & Kiely, 2011).
137. Choice of measurement methodology can also influence the absolute quantification of serum concentrations of 25(OH)D (see next section). In addition there is ongoing debate regarding thresholds for serum 25(OH)D concentration that indicate vitamin D deficiency, inadequacy, sufficiency.
138. As serum 25(OH)D concentrations increase, serum PTH falls. For this reason, the threshold concentration above which there is no further suppression of PTH has been suggested as a biochemical marker for distinguishing adequate vitamin D status from inadequacy/insufficiency; however, this is much debated (IOM, 2011; Holick *et al*, 2011). While circulating PTH concentration can be indicative of clinical vitamin D deficiency, its use as a marker of vitamin D status is hindered by a number of uncertainties, such as the

¹⁹ C-reactive protein (CRP) is an acute phase protein produced by the liver; plasma/serum concentrations rise in response to inflammation.

nature of the 25(OH)D–PTH relationship, and concentrations of PTH which may have adverse effects on bone health (IOM, 2011). In addition, serum PTH concentration varies widely within and among individuals and appears to be dependent upon age, race, ethnicity, body composition, renal function, as well as dietary intake of calcium and phosphorus.

139. The ratio of serum 24,25(OH)₂D to 25(OH)D concentration has also been suggested as an indicator of vitamin D deficiency (Wagner *et al*, 2011; Cashman *et al*, 2014; Kaufmann *et al*, 2014); however, more research is needed about the utility of this ratio over that of measuring serum 25(OH)D concentration.

Assessment of vitamin D exposure

140. Assessment of habitual dietary intake of vitamin D and sunshine exposure can be useful additions to the biochemical assessment of serum 25(OH)D concentration but both have their limitations.
141. Exposure to solar UV light has been assessed both directly and indirectly. Direct assessment methods include use of UV dosimeters which can be incorporated into badges, bracelets, or watches. Indirect methods include self-reported questionnaires and diaries. Both approaches have their strengths and weaknesses (reviewed in McCarty, 2008).
142. Accurate assessment of habitual vitamin D intake (including both vitamin D and 25(OH)D in foods) can be hindered by lack of up-to-date and accurate food composition databases for vitamin D. In addition to optimising analysis of raw foods or commodities, consistent monitoring of the levels of vitamin D (and correct identification of the vitamers D₂ and D₃) added to manufactured foods including supplements is also required to maintain currency of the databases (Cashman & Kiely, 2011). Another difficulty is that there are few naturally rich sources of vitamin D and these are consumed relatively infrequently; this means their consumption could be missed by some dietary assessment methods (e.g., food diaries recording all food consumed over a few days or week) if they were not consumed in the recording period.

Measurement of serum 25(OH)D concentration

143. Serum concentration of ‘total’ 25(OH)D (i.e., comprising the sum of 25(OH)D₂ and 25(OH)D₃) is used diagnostically and clinically as well as in the derivation of dietary reference values for vitamin D. However, it may also be useful to know the serum concentrations of these two metabolites separately in population studies, particularly national nutrition and health surveys, since use of both vitamin D₂ and vitamin D₃ is widespread; however, some immunoassays do not detect 100% of 25(OH)D₂ (see below).
144. A C-3 epimer²⁰ of 25(OH)D, which has been identified in infant, paediatric and adult populations (Singh *et al*, 2006; Strathmann *et al*, 2012), including in 96% of a nationally representative sample of adults (Cashman *et al*, 2014b) has no known function. If the C-3 epimer of 25(OH)D is found to have biological activity, it might need to be quantified (de la Hunty *et al*, 2010). Even if it is not shown to have biological activity it may be important to account for its contribution to total 25(OH)D concentration in samples from certain life-stage groups (e.g., neonates) where it has been reported to contribute 9-61% (median, 24%; mean, 28%) to the total 25(OH)D concentration (Singh *et al*, 2006). The C-3 epimer is included in many of the assay methods in current use.
145. Serum 24,25(OH)₂D₃ concentration can range from 2% to 20% of total serum 25(OH)D concentration (Bosworth *et al*, 2012) and has been shown to increase in direct proportion to that of serum 25(OH)D₃ concentration (Kaufmann *et al*, 2014; Cashman *et al*, 2014).
146. The impact of pre-analytical factors (e.g., serum versus plasma, fasting versus non-fasting state, or time of day) on serum 25(OH)D concentration is not well defined.

²⁰ Epimers have identical molecular structure but differ in stereochemical configuration.

147. While a variety of methods are available to determine serum 25(OH)D concentrations, each has presented technical problems and each has its advantages and disadvantages that need consideration when evaluating the data. These considerations impact on the choice of methodology for measuring vitamin D exposure as not all are able to discriminate between 25(OH)D₂, 25(OH)D₃, 24,25(OH)₂D₃ or the C-3 epimer of 25(OH)D.
148. The two most common types of assays for measuring serum 25(OH)D concentration are: antibody-based methods, which use a kit or an automated clinical chemistry platform; and liquid chromatography (LC)-based methods with either UV or mass spectrometric (MS)-detection. While both types of assay provide a measure of total serum 25(OH)D concentration, the LC-based methods (depending on system configurations, conditions of use and performance, duration of run times etc.) allow for separate estimation of 25(OH)D₂ and 25(OH)D₃ concentrations (and in some cases, the C-3 epimer and 24,25(OH)₂D₃) from serum samples.
149. With antibody-based methods, various commercial assays differ because of the nature of the antibody used. Some claim as an advantage, the fact that they do not discriminate between 25(OH)D₂ and 25(OH)D₃ (Hollis & Napoli, 1985) while others underestimate the 25(OH)D₂ component and therefore provide correction factors to compensate for high 25(OH)D₂ content (IOM, 2011). Some manufacturers of the antibody-based assays report >100% cross-reactivity of the antibody with 24,25(OH)₂D₃ which can contribute to a positive bias in serum 25(OH)D concentrations relative to LC-tandem MS methods (Cashman *et al*, 2015) (described below). It is important to recognise that most samples collected over the past 20-30 years, which have provided the majority of current evidence relating serum 25(OH)D concentration to health outcomes, have been analysed using antibody-based assays.
150. LC-based assays which use a tandem mass spectrometer (LC-MS/MS) allow discrimination between 25(OH)D₂ and 25(OH)D₃ and other compounds by their unique molecular masses and mass fragments (Makin *et al*, 2010). Since these methods use short LC retention times, and in some cases automated robotic extraction and LC separation steps and computerised MS systems, they can be made relatively operator-free and provide high throughput. Their potential advantages also include high specificity, high sensitivity, and better reproducibility (< 10%). The consensus among analysts is that LC-MS/MS assays will become the '*gold standard*' for assay performance in the future (de la Hunty *et al*, 2010; IOM, 2011).

Standardisation of the measurement of serum 25(OH)D concentration

151. While assay performance has been a concern of analysts and clinicians in the vitamin D field for some time, the role of standard reference materials and inter-laboratory collaboration and quality assurance schemes is an important aspect of overcoming the challenges that the assay methodologies present.
152. The *Vitamin D External Quality Assurance Scheme* (DEQAS²¹) serves as a quarterly monitor of performance of analysts and 25(OH)D analytical methods for approximately 700 laboratories worldwide (Carter *et al*, 2010). DEQAS has published performance reports regularly over the past decade, which suggest some method biases in terms of accuracy and precision as well as variability as high as 15-20%. However, some skilled analysts can perform better than this with a coefficient of variation less than 10%. The introduction of the National Institute of Standards and Technology (NIST) reference standards, calibrated using a "validated" LC-MS/MS method (Phinney, 2009) suggests that the variability of all methods will be improved in the future and that an improvement is already occurring (Carter & Jones, 2009).
153. The issue of international standardisation of serum 25(OH)D measurement is also being progressed by the Vitamin D Standardization Program (VDSP), a collaborative initiative between the Office of Dietary Supplements of the National Institutes of Health, the Centers for Disease Control and Prevention, the NIST

²¹Based at Charing Cross Hospital, London, UK.

and a number of the national health surveys around the world (VDSP, Federal Register, 2011; Binkley & Sempos, 2014). The International quality assurance/collaboration schemes, such as DEQAS and VDSP as well as existing and next generation standard reference materials for 25(OH)D, will further help limit inter-laboratory assay-specific differences in this status marker.

Interpretation of measures of serum/plasma 25(OH)D concentration

154. The normal range of serum 25(OH)D concentration is broad and the lower limit can vary among populations (Weaver & Fleet, 2004). There is considerable and continuing debate on the suggested threshold (cut off) for serum/plasma 25(OH)D concentration used to define low vitamin D status, which has ranged between 12.5 and 120 nmol/L (5 and 48 ng/ml) (Zittermann, 2003). This is principally because different functional endpoints/outcome indicators have been suggested to have different serum 25(OH)D concentration thresholds, although there has also been disagreement over the appropriate threshold concentration for a specific functional endpoint(s).
155. For example, the IOM DRI committee, using bone health as the basis for developing dietary reference intakes for vitamin D, proposed a serum 25(OH)D concentration of 40 nmol/L as the median value above which approximately half the population might meet its vitamin D requirement (in terms of bone health and below which half might not) and 50 nmol/L as the concentration that would meet the requirement of nearly all (i.e. 97.5%) '*normal healthy persons*'. The IOM DRI committee also suggested that: individuals are at risk of deficiency at serum 25(OH)D concentrations < 30 nmol/L; some, but not all, individuals are potentially at risk for inadequacy at serum 25(OH)D concentrations from 30 up to 50 nmol/L; and practically all individuals are sufficient at concentrations of 50 nmol/L and above.
156. In contrast, the Endocrine Society Task Force on Vitamin D (Holick *et al*, 2011) suggests that '*individuals should be identified as vitamin-D-deficient at a cut-off level of 50 nmol/L serum 25(OH)D*'. and '*to maximise the effect of vitamin D on calcium, bone, and muscle metabolism*', serum 25(OH)D concentration '*should exceed 75 nmol/L*'.
157. While both the IOM DRI committee and the Endocrine Society Task Force appeared to agree that there was insufficient evidence for any non-skeletal effects of vitamin D, others have suggested serum 25(OH)D thresholds between 50-120 nmol/L in relation to benefits on non-skeletal outcomes (Zittermann, 2003; Holick, 2004).
158. The wide variation in serum 25(OH)D concentrations, made using different methods and in different laboratories, should be taken into account in the interpretation of studies that have examined the relationship between serum 25(OH)D concentration and health outcomes.

5. Relationship between vitamin D exposure (from diet & skin synthesis) and serum 25(OH)D concentration

159. Humans have two routes of exposure to vitamin D:

- i. Vitamin D₃ derived from synthesis in human skin on exposure to UVB containing sunlight.
- ii. Dietary exposure through consumption of vitamin D₂ and D₃ in the form of naturally occurring foods, fortified foods and dietary supplements. Some animal derived foods may contain small amounts of 25(OH)D₃ in addition to vitamin D₃.

Relationship between vitamin D intake and serum/plasma 25(OH)D concentration

160. The relationship between dietary exposure to vitamin D and serum 25(OH)D concentration could be considered as the response of serum 'total' 25(OH)D concentration (i.e., summation of 25(OH)D₂ and 25(OH)D₃) to altered intake of vitamin D₂ and/or D₃ (plus 25(OH)D₃ in some cases). There are a number of considerations which may impact on this relationship.
161. Vitamin D₂ and D₃ differ only in their side chain structure and both elevate serum total 25(OH)D concentration (Seamans & Cashman, 2009). However, there is disagreement on whether both vitamins are equally effective in raising and maintaining serum total 25(OH)D concentration (see paragraphs 71-75, chapter 2).
162. Data suggest that per µg of vitamin D compound consumed, 25(OH)D₃ (a minor dietary form) is approximately 5-times as effective as vitamin D₃ in elevating serum 25(OH)D₃ concentration (Cashman *et al*, 2012). This needs to be accounted for when deriving total vitamin D activity estimates for some foods of animal origin (particularly in meats and eggs).
163. It has been suggested that efficient absorption of vitamin D is dependent upon the presence of fat in the intestinal lumen (Weber, 1981). Some physiological factors may also impact on the response of serum 25(OH)D concentration to vitamin D intake. For example, in a study of healthy young adult men (n=116; age 28 ±4 y), Barger-Lux *et al* (1998) reported that larger BMI or higher baseline serum 25(OH)D concentration, the smaller the rise in 25(OH)D concentration for any given dose of vitamin D. Forsythe *et al* (2011), using data from RCTs (Cashman *et al*, 2008; 2009), reported that BMI was negatively associated with change in serum 25(OH)D concentration following supplementation in older (≥ 64 y; n=109) but not younger (20-40 y; n=118) adults. The degree of adiposity (overweight versus obesity) may also be an important factor. Barger-Lux *et al* (1998) also observed that the higher the baseline serum 25(OH)D concentration, the smaller the achieved concentration in response to a given dose of vitamin D. However, a meta-regression analysis reported that baseline serum 25(OH)D concentration did not influence the response of serum 25(OH)D concentration to vitamin D (IOM, 2011).
164. Despite these considerations, the relationship between vitamin D (not distinguishing between vitamin D₂ or D₃) intake and serum/plasma 25(OH)D has been described. While a number of RCTs have reported the response of serum 25(OH)D concentration to increased vitamin D intake (by supplementation), there was great variability and many were not dose-response trials. Exploratory meta-regression analyses of RCT data, 16 trials in adults (Cranney *et al*, 2007) and 36 trials in children and adults (Seamans & Cashman, 2009) reported that for each additional 1 µg (40 IU) of vitamin D consumed, serum 25(OH)D concentrations increased by 0.64 and 0.53 nmol/L, respectively. The RCTs were from many different countries and conducted in different seasons. The estimates are in good agreement with the often quoted slope estimate (0.7 nmol/L per 1 µg vitamin D) from the regression equation developed in a dose-response study among healthy young men in Omaha, Nebraska, USA (latitude, 41.2°N), which assessed changes in

serum 25(OH)D concentrations in response to extended oral dosing with vitamin D₃ over an extended winter period (Heaney *et al*, 2003).

165. These estimates assume a linear relationship between vitamin D intake and serum 25(OH)D concentration, which may be inappropriate in certain circumstances. At an increased concentration of circulating vitamin D (~15 nmol/L; equivalent to a daily input from all sources [diet and sun] of about 50 µg), the hepatic CYP2R1 enzyme (responsible for activating vitamin D to 25(OH)D) becomes saturated and the reaction switches from first to zero order (Heaney *et al*, 2008). Therefore, the rapid increase in serum 25(OH)D concentration with increasing serum vitamin D₃ concentration, which occurs at the lower end of the range, becomes slower at higher circulating concentrations of vitamin D. This means that the response of serum 25(OH)D concentration to vitamin D intake is not linear over an extended vitamin D intake range. An analysis of 64 vitamin D RCTs (Aloia *et al*, 2008) showed that the slope response of serum 25(OH)D concentration to increasing doses of oral vitamin D flattens off at a dose of 35 µg/d (1400 IU).
166. The IOM also reported a steeper rise in serum 25(OH)D concentration at vitamin D doses < 25 µg/d (1000 IU) and a slower more flattened response at doses ≥ 25 µg/d (1000 IU) regardless of baseline intake or serum 25(OH)D concentration (IOM, 2011). Therefore, in its meta-regression analysis of data from selected RCTs, the IOM used a curvilinear relationship (achieved by a natural logarithm [Ln] transformation of serum 25(OH)D concentration versus total vitamin D intake) to allow for a more blunted response of serum 25(OH)D at intakes above 25 µg/d (1000 IU). The 95th percentile of total vitamin D intake in national nutrition surveys in Europe is generally less than 15 µg/d (600 IU) (Flynn *et al*, 2009) so the intake range for many populations is likely to lie where the intake-status relationship is more linear. The shape of the intake-serum 25(OH)D relationship (linear versus curvilinear) has an important bearing on estimating the vitamin D intake required to achieve a specified serum 25(OH)D concentration (particularly those below 50 nmol/L) (Cashman *et al*, 2011b).
167. A number of European (51-60°N) winter-based, dose-related RCTs which used supplemental doses of vitamin D between 0-20 µg/d (800 IU) (in the linear part of the response curve) have reported vitamin D-serum 25(OH)D concentration slope estimates of 1.55-2.43 nmol/L increment per 1 µg vitamin D (Cashman *et al*, 2008, 2009, 2011; Viljakainen *et al*, 2008); this is much higher than that of Heaney *et al* (2003) and the two meta-analyses estimates (Cranney *et al*, 2007; Seamans & Cashman, 2009; see paragraph 164 above).
168. The meta-regression analyses by Cranney *et al* (2007) and Seamans and Cashman (2009), of the response of serum 25(OH)D concentration to vitamin D intake, used data from RCTs conducted in the winter-time when the influence of UVB sunlight-derived dermal synthesis of vitamin D is minimised. At times of the year when UVB sunlight is sufficient for dermal production of vitamin D, the absolute percentage of serum 25(OH)D concentration arising from cutaneous synthesis versus oral intake of vitamin D cannot be clearly specified. For example, the IOM reported that a similar meta-regression analysis on data from winter-based RCTs conducted in the latitude band 40 to 49.5°N (where assumption of minimal sun exposure may not be as fully met compared to latitudes from RCT > 49.5°N or S (which were used for derivation of the current US RDA values) yielded quite different regression equations (resulting in lower RDA estimates), highlighting the impact of UVB exposure (in this case only that arising during the extended winter in these lower latitude regions) on the estimated dietary vitamin D requirement values. The IOM, therefore, used data from RCTs at higher latitudes to ensure as little contribution from endogenous production as the evidence would allow.

Relationship between vitamin D intake and serum 25(OH)D concentration in African Americans

169. Findings from RCTs in the USA, which have examined the effect of vitamin D supplementation on African Americans are conflicting (Gallagher *et al*, 2013; Gallagher *et al* 2014; Ng *et al*, 2104). Based on their

findings from a 4-arm RCT (placebo, 25, 50, or 100 µg/d vitamin D₃ for 3 months) Ng *et al* (2014) estimated that 41 µg/d of vitamin D was required to maintain winter 25(OH)D concentrations > 50 nmol/L in 97.5% of African American men and women (n=292; age, 30-80 y). This is almost twice the amount (15-20 µg/d) established by the IOM based on data from RCTs with white people. However, since the study did not include a group with white skin it is not certain that there are differences in requirements by skin type.

170. In contrast, Gallagher *et al* (2013) reported that the increase in serum 25(OH)D concentration after vitamin D₃ supplementation (placebo, 10, 20, 40, 60, 80, 100, or 120 µg/d for 12 months) in older African American women (n=110; mean age, 67±7.5 years) was similar to that observed in white women (n=163; mean age, 67±7.3 years) in a similarly designed RCT (Gallagher *et al*, 2012) and that 20 µg/day of vitamin D was required to maintain 25(OH)D concentration > 50 nmol/L in 97.5% of African American and white women.
171. Another RCT by the same group (Gallagher *et al*, 2014), which was conducted in younger white and African American women with serum 25(OH)D concentration ≤ 50 nmol/L (n=198; age 36.7±5.9 years) and who were assigned to placebo or vitamin D₃ (10, 20, 40 or 60 µg/d for 12 months), reported that mean baseline serum 25(OH)D concentration was lower in African American women (29.0±10 nmol/L) than the white women (36.4±11 nmol/L). However, as the absolute increase in serum 25(OH)D concentration after vitamin D supplementation was greater in African American women than in white women, the mean serum 25(OH)D concentration after 12 months was similar in both races at higher doses. It was estimated using mathematical modelling that 10 µg/d of vitamin D was required to increase 25(OH)D concentrations > 50 nmol/L in 97.5 % of the white women and between 20 and 40 µg/d of vitamin D was required to increase 25(OH)D concentrations > 50 nmol/L in 97.5 % of the African American women.

Relationship between UVB sunlight exposure and serum 25(OH)D

172. The relationship between skin exposure to UVB sunlight and the resulting serum 25(OH)D concentration is much less well defined because it is complicated by a number of factors (e.g., season, time of day, amount of skin exposed, skin pigmentation, use of SPF sunscreen). (See also chapter 3.)
173. It has been suggested that, compared to vitamin D formed in the skin, dietary vitamin D is less efficient at maintaining serum 25(OH)D concentrations (Haddad *et al*, 1993). This could be because vitamin D synthesised in the skin is primarily associated with DBP and slowly diffuses into the blood stream, gradually arriving at the liver (Fraser, 1983). In contrast, dietary vitamin D is associated with chylomicrons and low density lipoproteins which are readily taken up by the liver.
174. A systematic review which examined the effect of UVB exposure on serum 25(OH)D concentration identified 8 randomised trials (Cranney *et al*, 2007). Four trials evaluated the effect of natural sun exposure and 4 evaluated the effect of artificial UV exposure on serum 25(OH)D concentration. Study populations ranged from infants to older adults and interventions were variable, ranging from 1 MED to specified minutes of exposure to mJ/cm². A quantitative synthesis of the trials of UVB exposure and serum 25(OH)D concentration was not possible due to the heterogeneous study populations, the interventions (length and area of exposure; dose) and lack of complete data (Cranney *et al*, 2007).
175. Laboratory studies that have investigated the relationship between UVR exposure and vitamin D synthesis have typically used UVB phototherapy sources which also contain non-solar UVB radiation (< 295 nm) that is also very effective at vitamin D production. It is, therefore, difficult to make comparisons with solar UVR. A study that compared doses of natural solar UVR (April-September) with doses of artificial UVB radiation of hands and face reported a significant increase in serum 25(OH)D concentration with UVB from artificial sources but not with sunlight (Datta *et al*, 2012). It was estimated that UVB from a phototherapy source was at least 8 times more effective (in terms of erythemally equivalent exposure) than solar UVB.

176. Laboratory studies (Bogh *et al*, 2010) suggest an inverse relationship between baseline serum 25(OH)D concentration and response to UVB: i.e., the lower the baseline serum 25(OH)D concentration, the greater the response.
177. In an RCT which examined interactions between exposure dose²² and body surface area (Bogh *et al*, 2011), participants (n=92; age, 18-65 y) received 4 UVB exposures (0.75, 1.5 or 3.5 SED) at intervals of 2-3 days. All exposures were for 10 minutes, except in 10 participants who received 5 minutes exposure (n=5 in each group who received 0.75 & 1.5 SED to 24% body surface area). Increasing the exposed body surface area from 6% to 24% decreased the effect of increasing UVR dose (see Fig 4) and increasing the exposure dose from 0.75 to 3.0 SED decreased the effect of increasing body surface area (see Fig 5). These data suggest higher doses are needed if small areas of the body are exposed and that lower doses are adequate if larger body surface areas are exposed.
178. Chel *et al* (1998) reported that exposure of the lower back of older females (mean age, 85 y) residing in a nursing home in the Netherlands (52°N) to ½ MED (from artificial UVB; individual doses adjusted according to skin sensitivity) 3 times a week for 12 weeks increased serum 25(OH)D concentration by 42 nmol/L (median of 60 nmol/L at end of trial); a second group who received 10 µg/d (400 IU) of vitamin D₃ over the same period increased serum 25(OH)D concentration by 37 nmol/L (median, 60 nmol/L at end of trial).
179. The amount of sun exposure needed to generate 1 MED (or some fraction of) will depend on external factors as well as individual factors such as skin type and time spent outdoors. A UK group (University of Manchester) has examined and reported the efficacy of a dose range of simulated summer sunlight exposures in raising serum 25(OH)D concentrations in UK white-skinned adults (Farrar *et al*, 2011) and in adult of South Asian ethnicity (Farrar *et al*, 2011, 2013).
180. Holick (2001, 2004) has suggested that exposure of approximately 25% of body surface, 2-3 times per week, to 1/4 MED in spring to autumn is equivalent to an oral dose of 25 µg (1000 IU) vitamin D. For the UK, in people with skin types I to IV, this corresponds to exposure times of around 5-15 minutes in mid-summer and 15-60 minutes in mid-March and mid-September (Webb & Engelsen, 2006). Many solar recommendations to achieve and maintain serum 25(OH)D at specific concentrations are based on this guideline; however, it is difficult to extrapolate it to solar UVB exposure since it was derived from full body exposure to doses of artificial UVR radiation containing non-solar UVB.
181. Diffey (2010) developed a mathematical model to estimate changes in serum 25(OH)D concentration from sun exposure throughout the year using data and calculations for synthesis and decay of serum 25(OH)D concentration following a specific sun exposure and accounting for various factors (including time outside, month, available UV radiation in the UK, % skin exposure). The results from this model suggest that 10-20 minutes of daily sun exposure during summer months in the UK may achieve a maximum increase of 5-10 nmol/L in serum 25(OH)D concentration.

²² Exposure doses were given in SED units: 1 SED is equivalent to an erythemal effective radiant exposure of 100 Jm⁻²; typically the MED of a fair-skinned individual is about 2-3 SED.

FIGURE 6 - Relationship between the increase in 25(OH)D and the UVB doses of 0.75 SED, 1.5 SED and 3.0 SED. Red line, 24% body surface area ($P = 0.08$; $R^2 = 0.108$); green line, 12% body surface area ($P = 0.0004$; $R^2 = 0.35$); blue line, 6% body surface area ($P < 0.0001$; $R^2 = 0.48$)(data from Bogh et al, 2011).

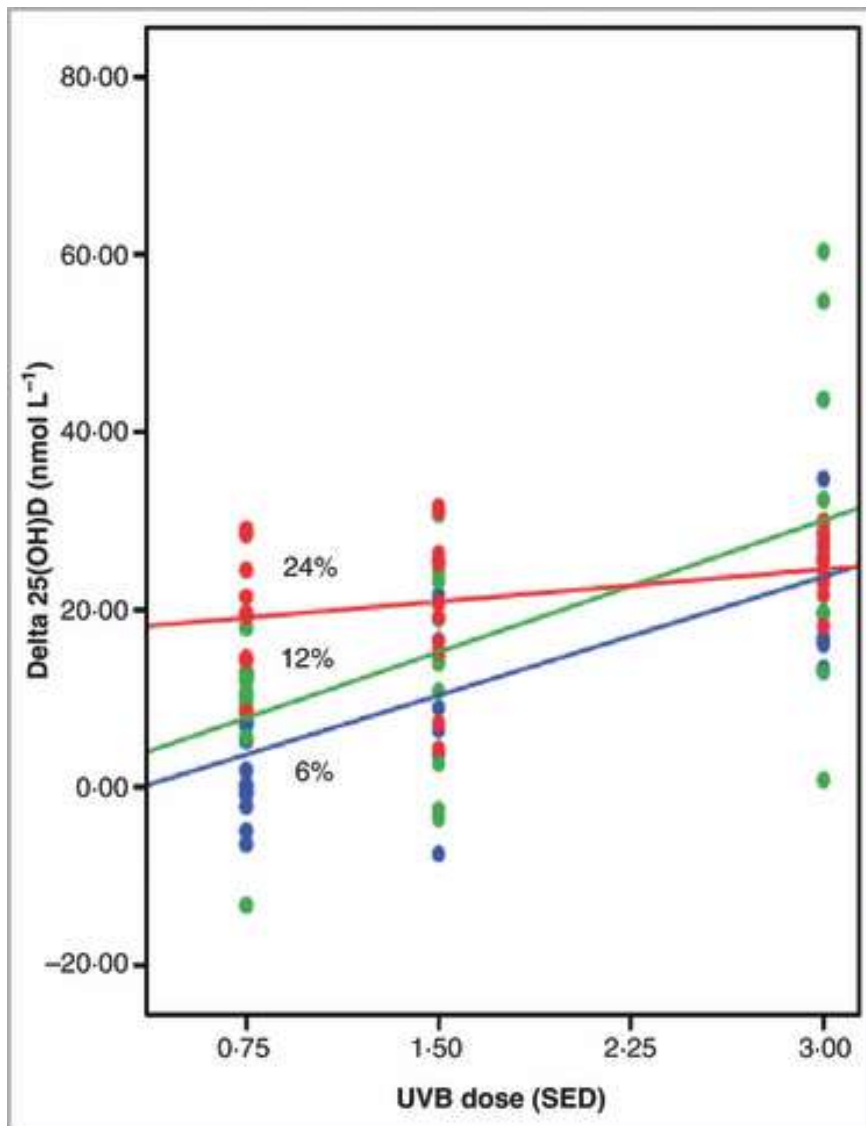
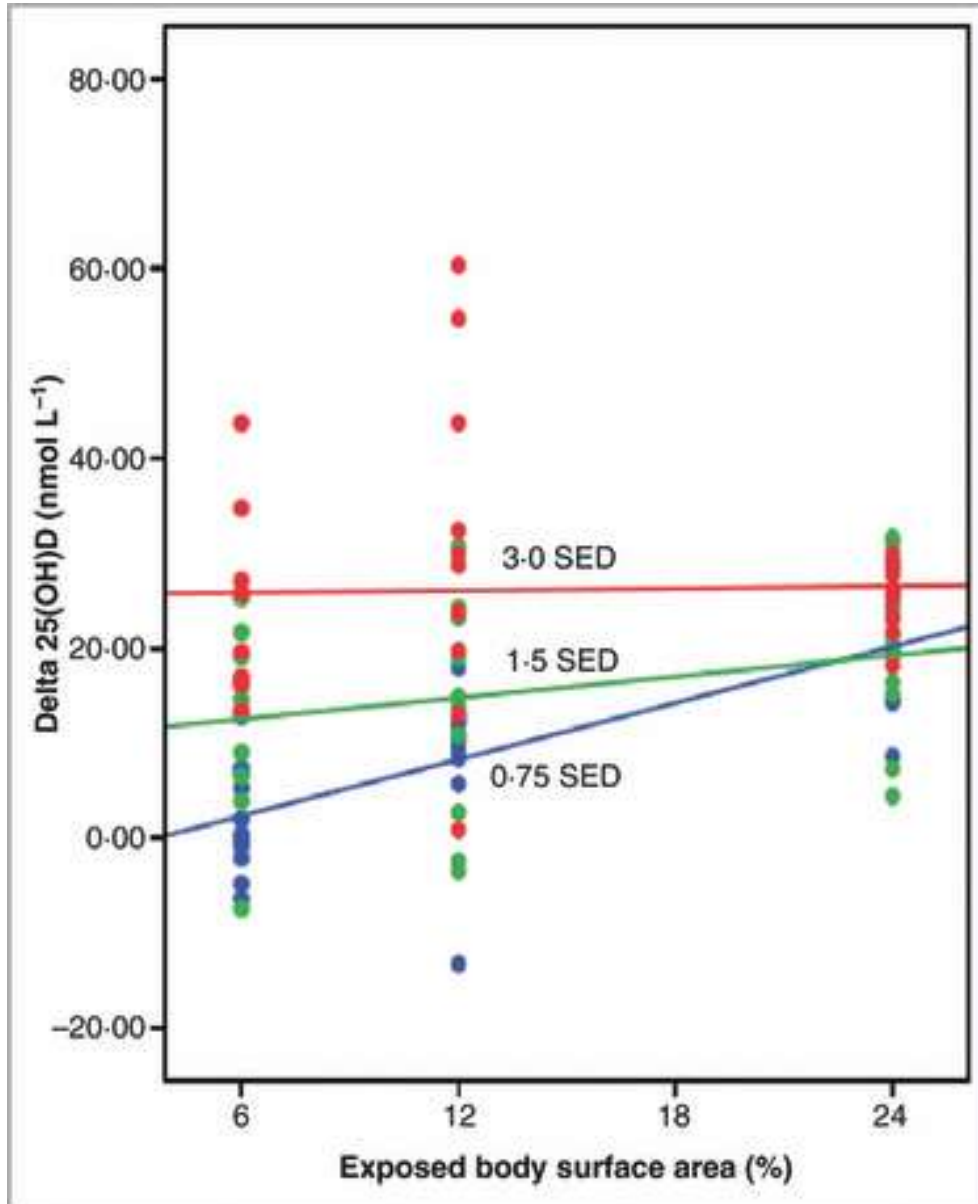


FIGURE 7 - Relationship between the increase in 25(OH)D and the exposed body surface areas of 6%, 12% and 24%. Red line, 3 SED ($P = 0.9$; $R^2 = 0.001$); green line, 1.5 SED ($P = 0.15$; $R^2 = 0.073$); blue line, 0.75 SED ($P < 0.0001$; $R^2 = 0.56$) (data from Bogh *et al*, 2011).



6. Vitamin D and health outcomes

182. The purpose of reviewing the evidence for a relationship between vitamin D and various health outcomes was to assess whether they might inform the setting of dietary reference values for vitamin D. The health outcomes examined were those considered to be of public health importance.
183. Serum 25(OH)D concentration represents exposure to vitamin D from UVB containing sunlight and from the diet. Skin synthesis, rather than diet, is the main source of vitamin D for most people. Consideration of evidence was largely confined to studies that compared health outcomes against serum/plasma 25(OH)D concentration since this reflects exposure to vitamin D from both sunlight and diet. Observational studies which only examined the relationship between vitamin D intake and health outcomes were not considered.
184. Consideration of the evidence was restricted to studies that examined whether vitamin D reduced the risk or incidence of specific health outcomes and not its effect as a therapeutic agent in reducing severity or progression of pre-existing disease; i.e., disease prevention rather than cure.

Process

185. The IOM report, *Dietary Reference Intakes for Calcium and Vitamin D*, was published in 2011. In order to inform their considerations, the IOM commissioned two evidence-based systematic reviews which were conducted by the Agency for Healthcare Research and Quality (AHRQ): AHRQ-Ottawa (Cranney *et al*, 2007); AHRQ-Tufts (Chung *et al*, 2009). The IOM report synthesised the evidence from the Ottawa and Tufts reviews and conducted its own literature search to update the AHRQ reviews.
186. In 2014, the AHRQ published an update of studies conducted since its 2009 review (Newberry *et al*, 2014). In general, the main findings did not differ from those of the earlier review (Chung *et al*, 2009) or the IOM update of the evidence.
187. Data included in the IOM report, together with evidence published since then, was considered by the SACN vitamin D working group (WG). A systematic methodology was not used in the literature searches to update the evidence. Instead, position papers on vitamin D and specific health outcomes were prepared by members of the WG, according to their expertise, which identified and summarised the key evidence published since the IOM report. The position paper and the original studies cited in the position papers provided the basis for discussions and judgements on the quality of the evidence at WG meetings. Evidence published up to September 2014 was considered in this draft report including the studies identified in the AHRQ update (Newberry *et al*, 2014).
188. Evaluation of the evidence was based on SACN's framework for risk assessment (2002; 2012) which recognises the contribution of different study types in making an overall assessment. The framework is based on an evidence 'hierarchy' which is used to judge the strength of the evidence according to study design, because each study design has its own strengths and weaknesses. In general, most weight is placed on randomised controlled trials (RCTs) and less weight on observational (non-intervention) studies. This is because observational studies are potentially subject to bias, confounding and reverse causality. However, it is not always feasible or ethically appropriate to conduct RCTs and/or this type of evidence may not be available. In the absence of RCTs, evidence from non-randomised intervention studies and prospective studies is given greater weighting than other study designs (case-control, cross-sectional and case reports).
189. In assessing the evidence on vitamin D and health outcomes, data from RCTs, then prospective studies, were preferred in terms of informing the setting of DRVs when these were available; however, the portfolio of evidence that was considered included data from other study types including case-control and

cross-sectional studies and case reports. Within in each study type, systematic reviews/meta-analyses, if available, were considered first and then any individual studies that contributed to or were published subsequent to the systematic reviews/meta-analyses. In some instances, where more than one systematic review/meta-analysis addressed the same health outcome, there was overlap in the primary data selected for inclusion.

190. For each of the potential health outcomes considered, the first step was to make a judgement on whether the evidence suggested a relationship between vitamin D supplementation or serum 25(OH)D concentration and the health outcome. If data were lacking or inconsistent for a specific health outcome, then that health outcome was not considered any further. If the evidence was suggestive of a relationship between a specific health outcome and vitamin D supplementation/serum 25(OH)D concentration then the data were examined further for evidence of threshold effect or whether a distribution of serum 25(OH)D concentrations or threshold serum 25(OH)D concentration associated with beneficial effects could be identified. An important limitation to this task, however, was that there is no clear consensus on the threshold serum 25(OH)D concentration used to define vitamin D deficiency or low status and cut-offs to characterise vitamin D deficiency or low status have vary across different studies. In most of the studies which considered whether there were threshold effects of serum 25(OH)D concentration on health outcomes, cut-offs were predefined according to different criteria for deficiency. As a consequence, these cut-offs were very insecure and made it difficult to assess if there was a dose response.

Potential sources of bias and confounding in studies of vitamin D and health outcomes

191. A number of factors need to be considered in assessing the evidence on vitamin D and health outcomes. As well as the general confounders in studies of diet and disease (such as smoking, alcohol, physical activity, medical treatment and social class), factors that affect cutaneous synthesis of vitamin D need to be taken into consideration; these include: season of year, latitude, skin exposure to sunlight, skin pigmentation, time of exposure, sun screen use, urban environment (can reduce/block sunlight), air pollution and cloud cover.
192. Although serum 25(OH)D concentration is a marker of exposure to vitamin D (from sunlight and the diet), a number of factors complicate its use in studies of the relationship between vitamin D and health outcomes. Since it reflects exposure to vitamin D, its concentration will be affected by factors that influence skin synthesis of vitamin D (see previous paragraph). Another important factor for consideration in observational studies is that people with a higher serum 25(OH)D concentration tend to be healthier than those with lower concentrations because of greater exposure to sunlight as a result of greater outdoor physical activity and/or a healthier diet and/or prophylactic use of supplements.
193. In addition to the variability which affects serum 25(OH)D concentration (e.g., time of day/time of year blood sample taken), concentrations can vary considerably (15-20%) depending on the type of assay used. There is also a lack of agreement between different laboratories using the same methods (de la Hunty *et al*, 2010). Serum 25(OH)D concentration may also decrease in response to acute inflammation which raises further concerns about its reliability as a marker of exposure since a low serum 25(OH)D concentration may simply reflect an underlying inflammatory state. (Problems relating to measurement of serum 25(OH)D concentration are considered in more detail in chapter 4.)
194. Serum 25(OH)D concentration is influenced by genetic variation and by physiological state; for example, the concentration is lower during periods of rapid bone growth. It is unclear whether this is because of physiological changes or because vitamin D supply is inadequate to meet requirements.
195. Serum 25(OH)D concentration is also inversely related to BMI. A lower concentration is more prevalent in overweight and obese individuals compared with normal weight individuals (Wortsman, 2000)

196. Another important limitation in many studies assessing the relationship between serum 25(OH)D concentration and health outcomes is the use of only one blood sample, because of individual variability of serum 25(OH)D concentration. A single measurement at baseline also does not allow evaluation of any impact of changes over time. There is also no standardised season for collecting blood samples.
197. All these factors have implications for the interpretation of studies, particularly observational studies, that have examined the relationship between serum 25(OH)D concentration and health outcomes.

Review of the evidence

198. Assessment of the evidence is divided into musculoskeletal and non-musculoskeletal health outcomes. Consideration of each health outcome includes a short summary of the IOM findings for that outcome.

Musculoskeletal health outcomes

Bone structure and metabolism

199. Bone is a composite material with an inorganic mineral component (69%) of calcium phosphate in the form of hydroxyapatite (99%), which provides it with hardness and rigidity, deposited around an organic matrix consisting of collagen (90%) and non-collagen structural proteins.
200. Bone is a highly specialised metabolically active tissue which provides both a structural function and a mineral reservoir for calcium and phosphorus. It is composed of an outer layer of dense and solid cortical (compact) bone which surrounds the marrow space and a lighter inner layer of trabecular (cancellous) bone with a mesh structure. Different bones and skeletal sites have different ratios of cortical to trabecular bone but, overall, the human skeleton comprises 80% cortical bone and 20% trabecular bone (Eriksen *et al*, 1994).
201. During the lifespan, bone undergoes processes of growth, modelling and remodelling (Clarke, 2008). Longitudinal and radial growth of bone occurs during childhood and adolescence. At maturity, bone stops growing in length but continues to grow in width and change shape in response to physiological influences or mechanical forces in a process known as *modelling*. *Bone remodelling* is a continuous lifelong process of replacement and repair, in which old bone is broken down (resorption) and new bone formed (formation or ossification), and which adapts the skeleton to physical stress (related to physical activity and load bearing) and to release ionised calcium and phosphate as required.
202. Bone cells involved in bone modelling and remodelling are osteocytes, osteoclasts and osteoblasts. Osteoblasts and osteoclasts, which originate in the bone marrow, are responsible for the processes of new bone formation and bone resorption, respectively. Osteoblasts synthesise osteoid (uncalcified pre-bone tissue) and facilitate its calcification; osteoclasts are phagocytic cells which remove bone tissue; and osteocytes, which are derived from osteoblasts, play a role in activation of bone formation and resorption (Datta *et al*, 2008). The activation process is regulated by mechanical forces, bone cell turnover, hormones (e.g., PTH), cytokines and local factors.
203. Bone mass accrual is rapid in the fetus and infants. It continues to increase during childhood at a slower rate until the adolescent growth spurt when it again undergoes rapid growth. During these periods of growth, bone turnover is very high and formation exceeds resorption leading to a net gain in bone mass. Peak bone mass is reached, typically, in the early 20s. In the young adult skeleton, bone formation and resorption is in approximate balance. With increasing age, the process of bone resorption predominates over bone formation leading to a net loss of bone mass. Bone mass later in life depends on peak bone mass reached at skeletal maturity and the subsequent rate of bone loss. The rate of bone loss is initially slow but, in women, accelerates rapidly in the first 4-8 years following menopause and then at a slower continuous rate throughout the rest of life (Riggs *et al*, 2002). The accelerated rate of bone loss is caused

by the sudden decline in oestrogen production by the ovaries at menopause. For men, bone loss is slow and continual; therefore, women generally lose more bone than men.

204. Bone strength depends primarily on bone mass which accounts for about 50-70% of bone strength (Pocock *et al*, 1987). Bone strength is also affected by bone geometry, cortical thickness and porosity and trabecular bone morphology. The main determinant of bone mass is genetics; however, hormones (calcium regulating hormones and sex hormones), lifestyle factors such as diet and physical activity can also influence bone mass. Nutritional deficiencies, particularly of calcium, vitamin D and phosphorus can lead to formation of weak, poorly mineralised bone.

Skeletal disorders

205. Insufficient vitamin D during growth leads to the development of rickets (vitamin D deficiency rickets). If diagnosed early, vitamin D supplementation can reverse the symptoms but if the skeletal deformities are widespread and significant, and growth plates have begun to mature, as in puberty, then it cannot. In the UK, a serum 25(OH)D concentration < 25 nmol/L is the current threshold used to define increased risk of rickets (DH, 1991). Other causes of rickets include inadequate calcium intake, although this is more common in developing countries, and inadequate phosphorus intake. Osteomalacia in adults, like childhood rickets, develops as a result of vitamin D deficiency. It commonly presents with severe aching in bone and muscles and proximal muscle weakness making standing up and walking difficult and painful and results in a marked waddling gait. Osteomalacia arises from a disorder in the physiological process of bone turnover where the mineralisation phase of bone remodelling is impaired. When vitamin D deficiency is implicated in the aetiology of osteomalacia there is usually evidence of secondary hyperparathyroidism. Osteomalacia can also be caused by kidney or liver damage since this will interfere with vitamin D metabolism.

Assessment of bone health

206. In studies which have examined factors influencing bone health, the most clearly defined and clinically relevant endpoint is bone fracture; in most studies, however, intermediate outcome measures are used to assess bone strength. Measurement of areal bone mineral density (BMD), the quantity of mineral present per given area of bone (g/cm^2), is the most common proxy measure of bone strength and fracture risk. The most widely used technique to measure BMD is dual energy x-ray absorptiometry (DXA) which has high reproducibility and low radiation dose (DH, 1998). Other techniques include quantitative computer tomography (QCT) which allows three-dimensional assessment of the structural and geometric properties of the skeleton but the equipment is expensive and the radiation dose is relatively high. Peripheral QCT (pQCT) has a much lower radiation dose and allows three-dimensional assessment of the lower arms and legs and volumetric measures of BMD (g/cm^3). Ultrasound methods are also used; however the clinical relevance of ultrasound bone measures is less well understood.
207. Although there is a relationship between BMD and fracture risk, the extent of the relationship is not clear. BMD measurements do not provide a complete assessment of bone strength; other factors that contribute include bone size, shape, architecture, and turnover (Amman & Rizzoli, 2003). Additionally, BMD obtained by single or dual-energy techniques, is an areal density measurement (g/cm^2) derived by dividing bone mineral content (BMC) by the scanned area of bone. It does not measure volumetric density of the bone or the mineralised tissue within the bone (Prentice, 1994). Since both BMC and BMD are influenced by the size, shape and orientation of the bone, this limits its use in cross sectional studies of factors influencing bone health unless adjustment is made for the confounding influence of size. Bone mineral measurements are more useful in prospective studies where changes are assessed over time.
208. Interpretation of bone health indices such as BMD and BMC in children is less clear. BMD is a less

informative measure of bone health than BMC because BMD partially corrects for attained size and therefore dilutes any possible relationships with skeletal growth. Various methods have been used to adjust areal BMD to more closely represent volumetric BMD especially at the spine, including calculation of bone mineral apparent density (BMAD) (Faulkner *et al*, 1995).

209. Biochemical markers associated with bone formation and resorption have also been used to assess bone health. Serum concentrations of osteocalcin, procollagen carboxy peptide, procollagen amino peptide and bone-specific alkaline phosphatase are validated indices of bone formation (DH, 1998). Markers of bone resorption are based on breakdown products of type I collagen in serum or urine and include pyridinium crosslinks of collagen (PYR and DPYR) and C-terminal crosslinks of type I collagen (CTX). Limitations of current biochemical markers of bone metabolism include lack of tissue specificity for bone and an inability to distinguish the metabolic activity of different skeletal compartments (Garnero, 2014).

Consideration of the evidence on vitamin D and musculoskeletal health outcomes

(See Tables 1-27, Appendix 1)

210. Evidence on vitamin D and the following musculoskeletal health outcomes was considered: rickets, osteomalacia, bone health indices (e.g. BMC/BMD, biochemical markers of bone turnover), fracture prevention, risk of falls and muscle health.
211. The data were considered by life stage since different musculoskeletal health outcomes are relevant to specific age groups. Evidence on vitamin D and bone health indices was considered across all life stages; muscle strength and stress fracture risk was considered in young adults; fracture prevention, risk of falls and muscle health were considered in adults > 50 y.
212. Since rickets can affect infants and children and osteomalacia can affect all adults, studies on rickets and osteomalacia usually include more than one life-stage group. Evidence on these two musculoskeletal outcomes was therefore not considered by life stage but separately and before consideration of other musculoskeletal health outcomes.

Rickets

IOM report

213. The IOM concluded that, overall, there was fair evidence for an association between low serum 25(OH)D concentrations and confirmed rickets but the evidence for a threshold serum 25(OH)D concentration above which rickets does not occur was inconsistent. Thirteen studies were identified which assessed the association between serum 25(OH)D concentration and rickets in infants and young children: 1 RCT; 4 before-after studies; 8 case-control studies. However, it was noted that many of the studies were from developing countries where calcium intake is low and could, therefore, be confounded by dietary calcium
214. Six studies (1 RCT; 3 before & after; 2 case-control) reported mean or median serum 25(OH)D concentrations below 30 nmol/L in children with rickets; the remaining studies reported mean serum 25(OH)D concentration above 30 nmol/L (range 36-50 nmol/L).
215. The IOM concluded that if calcium intake was adequate, the risk of rickets was increased at serum 25(OH)D concentration < 30 nmol/L.

Evidence considered

216. In order to identify a threshold serum 25(OH)D concentration associated with rickets, a range of studies was considered including those cited in, and published since, the IOM report. Studies referenced in the following COMA/SACN reports were also considered: *Dietary reference values for food energy and*

nutrients in the UK (DH, 1991); *Nutrition and bone health* (DH, 1998); and *Update on vitamin D* (SACN, 2007).

217. A total of 40 studies were identified which included measurements of serum 25(OH)D concentration in children with rickets (16 case reports; 6 observational studies, 8 before & after studies, 7 case control and 3 intervention studies). Individual baseline serum 25(OH)D concentration in the case reports (n=12) ranged from < 2.5 to < 50 nmol/L and mean/median serum 25(OH)D concentrations ranged between 5-44 nmol/L in observational studies (n=7), 9-50 nmol/L in the before and after studies (n=8), 6-42 nmol/L in case-control studies (n=7) and 14-38 nmol/L in intervention studies (n=3).
218. In most studies, information was not provided on the season in which the blood sample used to measure serum 25(OH)D concentration had been taken. It is therefore possible that the variability in serum 25(OH)D concentrations might be due to the time of year the samples were drawn.
219. Rickets with unknown aetiology, often with serum 25(OH)D concentration < 25 nmol/L, is usually defined as vitamin D deficiency rickets. Since, many of the studies on rickets were from developing countries, the findings could be confounded by low calcium intakes; however, only 5 studies identified reported calcium intakes. It was therefore not possible to ascertain whether the rickets was caused solely by vitamin D deficiency or by low calcium intake. Another limitation of studies on rickets include the correlation between serum 25(OH)D concentration and skeletal effect, since the time course is unknown. None of the studies obtained measurements of serum 25(OH)D concentration before the development of rickets; serum 25(OH)D concentrations in all studies was measured in children who presented with features of rickets.
220. In case reports, serum 25(OH)D concentrations are likely to be low because presentation at a hospital usually occurs at a more advanced stage of a disease. A greater range of concentrations is found in population studies. For example, a study in Australia (Munns *et al*, 2012) of children (n=398; age, 0.2-15 y) with vitamin D deficiency rickets (defined as serum 25(OH)D concentration < 50 nmol/L and alkaline phosphatase concentration > 222 IU/L and/or radiological rickets) reported that serum 25(OH)D concentrations ranged from 2 to 50 nmol/L (median, 28 nmol/L). Data from wrist x-rays (n=95) showed that 71% of these had signs of radiological rickets but median serum 25(OH)D concentration was not statistically different between cases with radiological rickets (median, 18 nmol/L; range 5-45 nmol/L) and those without (median, 20 nmol/L; range 8-45 nmol/L).
221. Overall, based on the evidence, there is no clear threshold serum 25(OH)D concentration below which rickets occurs; however, individual serum 25(OH)D concentrations were < 25 nmol/L (the current threshold for risk of rickets) in the majority of case reports and mean serum concentrations were < 25 nmol/L in the majority of studies considered.
222. Although the risk of rickets appears to be increased at serum 25(OH)D concentrations < 25 nmol/L, and rickets of unknown aetiology, where serum 25(OH)D concentration is < 25 nmol/L, is usually defined as vitamin D deficiency rickets; such concentrations are not diagnostic of the disease.

Summary - Rickets

223. Evidence on vitamin D and rickets is mainly observational and therefore subject to confounding. Since most studies did not report on calcium intake, it is not clear if rickets was caused solely by vitamin D deficiency or low calcium intake.
224. Serum 25(OH)D concentration in case reports ranged from < 2.5 to < 50 nmol/L and mean/median concentrations ranged between 5 and 50 nmol/L in other study types. Individual and mean serum 25(OH)D concentrations were < 25 nmol/L in the majority of studies examined.
225. Most studies did not provide information on time of year the blood sample was drawn for measurement which might explain some of the variability in serum 25(OH)D concentration associated with rickets.

Osteomalacia

IOM Report

226. Based on findings from a post-mortem analysis of bone biopsies (Priemel *et al*, 2010) the IOM concluded that all individuals were free of osteomalacia when serum 25(OH)D concentrations were > 50 nmol/L and that a significant increase in the number of people displaying the mineralisation defect was not observed until serum 25(OH)D concentrations were < 30 nmol/L. The study on which this conclusion was based (Priemel *et al*, 2010) was given considerable prominence in the IOM report and used to support a serum 25(OH)D concentration of 50 nmol/L as providing coverage against osteomalacia for 97.5% of the population. This study is considered below (see paragraphs 231-232).

Evidence considered

227. The majority of evidence on vitamin D and osteomalacia (from early 1940s to 2013) comprises case reports and many studies do not report serum 25(OH)D concentrations.

Observational studies

228. Gilfre *et al* (2011) examined the clinical manifestations and most frequent causes of osteomalacia in a group of patients in Spain (n=28; mean±SD age, 55±28 y) diagnosed with osteomalacia over a period of 20 years. Clinical data were obtained from a detailed review of medical records. Osteomalacia diagnosis was by bone biopsy and/or by Bingham & Fitzpatrick criteria²³. Mean (±SD) serum 25(OH)D concentration was 15 ± 5 nmol/L in patients with vitamin D osteomalacia.
229. Preece *et al* (1975) measured serum 25(OH)D concentration from patients of south Asian ethnic origin living in Glasgow (n=35) with overt rickets or osteomalacia (clinical & biochemical evidence & radiological confirmation). Serum 25(OH)D concentration was < 7.5 nmol/L in all patients and was undetectable (< 1.25 nmol/L) in 57% of the patients.

Case reports

230. Most of the evidence on osteomalacia is based on case reports in which serum 25(OH)D concentrations ranged from 4-20 nmol/L. The majority of the case reports are of patients of south Asian ethnic origin living in the UK.

Bone biopsy study (Priemel *et al*, 2010)

231. In Germany, Priemel *et al* (2010) carried out a post-mortem analysis of transiliac crest bone specimens obtained during autopsies of victims of accidents, assaults, suicides and other unnatural or unexpected causes (n=401 males, mean age 58.7±17 y; n=274 females, mean age 68.3±17.3 y) to assess the minimum

²³ Defined as two of the following: low Ca, low P, elevated total alkaline phosphatase, radiographic findings.

serum 25(OH)D concentration required to maintain bone health (blood samples were also taken at autopsy). Osteomalacia was defined as pathological osteoid accumulation (increase in osteoid volume per bone volume > 2%). While a minimum serum 25(OH)D concentration associated with mineralisation defects could not be identified, excess accumulation of osteoid was not found in any individual with a serum 25(OH)D concentration > 75 nmol/L.

232. However, the study has a number of limitations, including: the criteria used to define bone mineralisation, which are not universally accepted; tetracycline labelling, the preferred method for measuring bone formation, was not used; data were reported as scatter plots without further statistical analysis or adjustment for age (range, 20-100 years) and sex; variability is increased at serum 25(OH)D concentrations below 50 nmol/L but it is unclear how this should be interpreted because of the wide age range of the population; mean serum 25(OH)D concentrations appear to be very low for Germany (25 nmol/L in summer and 15 nmol/L in spring); information on calcium intakes, which might also affect bone mineralisation, was not available.

Summary – Osteomalacia

233. Evidence on osteomalacia is limited mainly to case reports in which serum 25(OH)D concentrations ranged between 4 and 20 nmol/L.
234. Out of 2 cross-sectional studies of patients with osteomalacia, mean serum 25(OH)D concentration was 15 (±5) nmol/L in 1 study and < 7.5 nmol/L for all patients in the other.

Other musculoskeletal health outcomes (beyond rickets and osteomalacia) by life stage

Pregnancy and lactation

Bone health indices

235. During pregnancy and lactation, a large amount of calcium is provided by the mother to the developing fetus and neonate (Kovacs, 2008). An important physiological change in pregnancy is the doubling in the rate or efficiency of intestinal calcium absorption; however, evidence from animal studies in vitamin D deficient rats and *Vdr*-null mice indicate that vitamin D is not required for this. Pregnancy induced adaptations to maternal calcium homeostasis seem to meet fetal requirements for calcium. Although skeletal resorption can also release calcium into the circulation, the evidence is mixed on whether the maternal skeleton contributes substantial amounts of calcium to the fetus (Kovacs, 2008).
236. The relationship between bone health indices and maternal serum 25(OH)D concentration during pregnancy is unclear which complicates interpretation of associations between serum 25(OH)D concentration and bone health indices during this time (Brannon & Picciano, 2011).
237. Physiological changes that occur during pregnancy increase serum concentrations of 1,25(OH)₂D and DBP; serum 25(OH)D concentrations, however, remain unaffected. The underlying mechanisms for these changes are not clearly understood (Brannon & Picciano, 2011).

IOM report

238. The IOM identified only 1 cohort study which included maternal BMD as an outcome and found no relationship between serum 25(OH)D concentration and post partum changes in BMD. The Committee concluded that there was insufficient evidence for an association between a specific serum 25(OH)D concentration and BMC or BMD.

Evidence considered since IOM report

Intervention studies

239. No RCTs investigating effects of vitamin D supplementation during pregnancy/lactation on markers of bone health (in the mother or infants) have been published since the IOM report.

Cohort studies

Maternal outcomes

240. A study in Turkey (Haliloglu *et al*, 2011) investigated the relationship between serum 25(OH)D concentration and CTX in women (n=30; age 28.1 ± 3.7 y) receiving supplemental vitamin D₃ (10 µg/400 IU/d) during pregnancy and lactation. Mean serum 25(OH)D concentration was $19.1 (\pm 7.4)$, $15.7 (\pm 7.2)$, $11.1 (\pm 9.0)$ and $7.0 (\pm 4.5)$ nmol/L during the 1st, 2nd and 3rd trimester and post partum period respectively. No correlation was found between serum 25(OH)D and CTX concentration in the 1st trimester but there was a negative correlation in the 2nd and 3rd trimesters and the postpartum period ($r = -0.47$, $p=0.048$; $r = -0.89$, $p < 0.0001$; $r = -0.88$, $p < 0.001$ respectively).

Fetal/newborn outcomes

241. Mahon *et al* (2010) investigated the association between maternal serum 25(OH)D concentration and fetal femur growth in pregnant women (n=424; age, 20-34 years) within a prospective longitudinal study in the UK. High resolution 3D ultrasound was used to measure: femur length; distal metaphyseal cross-sectional area; and the ratio of the two (known as femoral splaying index). Women with serum 25(OH)D concentration < 50 vs > 50 nmol/L had increased splaying of the distal metaphysis of the fetal femur. No differences were seen in femur length. The same group previously reported that children born to mothers with serum 25(OH)D concentration < 50 nmol/L during pregnancy exhibited deficits in BMC at 9 years of age (Javaid *et al*, 2006).
242. In a secondary analysis of biochemical/anthropometric/bone data of women (n=125) and infants in a calcium supplementation study in a rural area of the Gambia, West Africa (Prentice *et al*, 2009), no significant trends/relationships were found between maternal serum 25(OH)D concentration and infant birth weight/bone health measures (BMC, size adjusted BMC, bone width, bone area). None of the women, however, had a serum 25(OH)D < 50 nmol/L.
243. Viljakainen *et al* (2010) investigated associations between serum 25(OH)D concentrations of Finnish mothers (n=125; aged 20-40 years) and bone health of newborns. Two equal sized groups were defined using a serum 25(OH)D cut off concentration of 42.6 nmol/L (median value of individual means during 1st trimester and 2 days postpartum). Babies born to mothers above the median had 13.9% higher tibia BMC ($p=0.01$) and 16.3% higher cross-sectional area ($p = 0.02$) but there were no differences in BMD or bone turnover markers.
244. Young *et al* (2012) examined the relationship between maternal serum 25(OH)D concentration (and Ca intake) on fetal bone growth in pregnant adolescent girls (n=171; age ≤ 18 y) in the US. Measurements were taken at 26 weeks of pregnancy and at delivery. Fetal sonograms were taken up to three times across gestation. Maternal serum 25(OH)D concentration > 50 vs < 50 nmol/L was significantly ($p < 0.01$) associated with greater fetal femur length and humerus length z scores.
245. Dror *et al* (2012) investigated the relationship between maternal and cord serum 25(OH)D concentrations and bone specific alkaline phosphatase (BSAP) and whole body BMC in newborns in a multi-ethnic US population (n=80 mother-infant pairs). Cord serum BSAP concentration was inversely correlated with infant whole body BMC and with cord serum 25(OH)D concentration but there was no association between cord serum 25(OH)D concentration and whole body BMC.

Summary – Bone health indices (pregnancy & lactation)

246. One small cohort study published since the IOM report found a negative correlation between maternal serum 25(OH)D concentration and a marker of bone resorption (CTX concentration) in the 2nd and 3rd trimester of pregnancy and the postpartum period.
247. Out of 5 cohort studies, 4 show a positive association between maternal serum 25(OH)D concentration and various indices of bone health in the fetus/newborn. Three studies chose pre-determined cut-offs to define vitamin D 'deficiency' (< 50 nmol/L in 2 studies and 42.6 nmol/L in 1 study).

Infants (up to 12 months)

Bone health indices

IOM report

248. The IOM reported inconsistent evidence for an association between serum 25(OH)D concentration and BMC in infants. Out of 2 RCTs examining the effects of vitamin D supplementation on BMC (Greer *et al*, 1982; Zeghoud *et al*, 1997), 1 reported no effect of an increase in serum 25(OH)D concentration on radial bone mass while the other reported a transient increase of BMC in the supplemented group compared with the unsupplemented group at 12 weeks but not at 26 weeks. Evidence from case control studies suggested an association between greater whole body BMC and higher serum 25(OH)D concentration.

Evidence considered since IOM report

Intervention studies

249. A study in South Korea (Kim *et al*, 2010) examined the effect of daily vitamin D supplementation (10 µg/400 IU for 12 months) on BMD in breast-fed infants (n=74) at 6 and 12 months of age. Vitamin D supplementation significantly increased serum 25(OH)D concentration but not BMD. However, there are a number of uncertainties in this paper including use of BMD rather than BMC or BMAD in growing children and data are not provided on power calculations for the sample size required to detect a bone density difference with treatment.
250. A study in India (Kumar *et al*, 2011) investigated the effect of vitamin D₃ supplementation (35 µg/1400 IU/wk for 6 months) on growth (secondary outcome²⁴) in low birth-weight term infants (n=2070; age ≤ 48 hours). After 6 months, the Mean serum 25(OH)D concentration was 55 nmol/L in the supplemented group and 36 nmol/L in the placebo group. Vitamin D supplementation significantly increased z scores at 6 months for weight (p=0.026), length (p=0.014) and arm circumference (p = 0.033) and significantly reduced the proportion of children with stunted growth (p=0.018). However, findings from this study should be interpreted with caution since there was a large loss to follow-up²⁵ and, since it was conducted in undernourished low birth weight infants, the findings may not be applicable to normal weight infants in the UK.
251. Abrams *et al* (2012) evaluated the effects of daily vitamin D₃ supplementation (10 µg/400 IU for 3 months) on BMC/BMD in Hispanic and non-Hispanic white infants (n=49; age, one week) in Texas, USA. Serum 25(OH)D concentration was significantly lower in Hispanic compared to non-Hispanic infants at birth (p=0.013) and after 3 months supplementation (p=0.014). There were no significant relationships between cord serum 25(OH)D concentration and BMC or BMD in the 1st week of life or after 3 months supplementation. Key uncertainties in this study were the small sample size (and likely lack of statistical power) and the short time span for observing a difference in bone health indices.

²⁴ Primary outcomes were mortality and morbidity.

²⁵ Anthropometric data were available for only 62% of original sample.

252. Holmlund-Suila *et al* (2012) evaluated the effects of different daily supplemental doses of vitamin D₃ (10 µg/400 IU; 30 µg/1200 IU; or 40 µg/1600 IU) on bone strength (evaluated by pQCT) in infants from age 2 weeks to 3 months (n=113). There were no significant correlations between serum 25(OH)D concentration and pQCT parameters.

Summary – Bone health indices (Infants)

253. Evidence for an effect of vitamin D supplementation on indices of bone health in infants is inconsistent. Out of 4 RCTs, 3 reported no significant effect of vitamin D supplementation on bone health indices (BMC, BMD, pQCT). One RCT reported positive effects of vitamin D supplementation on growth in under-nourished low birth weight infants in India; however, findings from this study may not be applicable to the UK population.

Children (1-3 y)

Bone health indices

254. The IOM did not consider this age group separately.
255. No intervention or cohort studies examining the relationship between serum 25(OH)D concentration and BMD/BMC in this age group could be identified.
256. A cross-sectional analysis of children in Canada (n=488; age, 1.8-6 y) reported that a plasma 25(OH)D concentration > 75 compared to < 75 nmol/L was significantly related to higher BMC and areal BMD at the forearm and whole body but not at the lumbar spine (Hazell *et al*, 2015).

Summary – Bone health indices (Children, 1-3y)

257. One cross-sectional study reported an association between serum 25(OH)D concentration > 75 nmol/L and higher BMC/BMD at the forearm and whole body but not at the lumbar spine.

Children (4-8 y) and adolescents

Bone health indices

IOM report

258. In the IOM report early childhood was defined as 4-8 years and puberty/adolescence was defined as 9-13 years and 14-18 years. The IOM concluded that there was fair evidence of an association between serum 25(OH)D concentrations, baseline BMD and change over time in BMD or BMD indices; however RCTs did not confirm a consistent benefit of vitamin D supplementation on BMD.

Evidence considered since IOM report

Systematic review and meta-analysis

259. A systematic review and meta-analysis of 6 intervention studies (Winzenberg *et al*, 2011) examined the effect of vitamin D₃ supplementation on bone density in children and adolescents (n=884; age, 8-17y). Mean baseline serum 25(OH)D concentration ranged from 17.7-49.5 nmol/L. Overall, vitamin D supplementation had no significant effects on BMC or BMD of the hip, forearm, lumbar spine BMD. When the meta-analysis was confined to studies in which mean baseline serum 25(OH)D concentration was < 35 nmol/L, the effect on total body BMC was significant (p=0.04; 3 studies; n=413) and bordering on significance for lumbar spine BMD (p=0.05; 2 studies; n=189), equivalent to a 2.6% and 1.7% greater change from baseline with supplementation.

260. There are a number of limitations in this meta-analysis which make it difficult to interpret the results: the studies were heterogenous in terms of sample size and ethnicity; 1 study administered vitamin D fortified milk rather than vitamin D supplements (Du, 2004); sub-group analysis for effects of vitamin D supplementation by mean baseline serum 25(OH)D concentration arbitrarily selected a cut-off of 35 nmol/L based on the distribution of data; therefore, effects in those with mean baseline serum 25(OH)D concentrations in any range below the selected cut-off were not considered. The effects of puberty on serum 25(OH)D concentrations were also not considered. Since the rate of bone accretion varies throughout puberty and by sex, higher calcium requirements during this time (due to increased growth velocity) might lead to greater 25(OH)D utilisation.

Intervention studies

261. Park *et al* (2010) reported no effect of daily vitamin D₃ supplementation (25 µg/1000 IU) on calcium absorption or skeletal retention in girls (n=11; age 12-14 y). Calcium excretion in the supplemented group increased by 33% but it is not clear if this was an adverse effect of supplementation or a homeostatic response to an increase in calcium absorption.
262. Molgaard *et al* (2010) reported that vitamin D₃ supplementation (5 or 10 µg/d; 200 or 400 IU/d) for 12 months had no effect on indices of bone health in girls (n=221; age 11-12 y) except on a subgroup with the FF VDR (but not in the Ff or ff VDR) genotype in which vitamin D supplementation increased whole body BMD (p=0.007) and BMC (p=0.048), indicating an influence of VDR genotype.
263. Ghazi *et al* (2010) found no effect of vitamin D₃ supplementation (1250 µg/50,000 IU) administered monthly (equivalent to 40 µg/1600 IU per day) vs bimonthly (equivalent to 20 µg/800 IU per day) vs placebo for 6 months on a marker of bone resorption (CTx) in boys and girls (n=210; age 14-20 y).
264. Ward *et al* (2010) reported no effect of vitamin D₂ supplementation (4 doses of 3750 µg/150,000 IU over 1 year) on BMD and BMC in adolescent girls (n=69; aged 12-14 years; 88% of south Asian origin).
265. A pilot RCT in India (Khadilkar *et al*, 2010) investigated the effect of vitamin D supplementation on size adjusted bone area and BMC in underprivileged adolescent girls (n=50; age, 14-15 y) randomised to receive either vitamin D₂ (7500 µg/300,000 IU) or placebo 4 times/y for 1 year; all participants also received calcium (250 mg/d). Median post supplementation serum 25(OH)D concentration was 75.2 (64.2-85.5) nmol/L in the intervention group and 28.1 (16.7-34.0) nmol/L in the placebo group. No significant difference was reported in bone outcome measures in the two groups.

Summary – Bone health indices (children, 4-8 y and adolescents)

266. A systematic review and meta-analysis reported a significant positive effect of vitamin D supplementation on total body BMC when baseline serum 25(OH)D concentration was < 35 nmol/L. However these findings should be interpreted with caution because of a number of limitations in the data and because the 35 nmol/L cut-off was arbitrarily selected based on the distribution of data.
267. The majority of subsequent intervention studies suggest no beneficial effect of vitamin D supplementation on bone health indices in children and adolescents. Out of 5 studies, none found an effect of vitamin D supplementation on bone health indices.

Muscle strength and function

IOM Report

268. Muscle strength and function in children and adolescents was not considered.

Evidence considered since IOM

Intervention studies

269. Ward *et al* (2010) examined the effect of vitamin D₂ supplementation (4 doses of 3750 µg/150,000 IU over 1 year) on muscle function in adolescent girls (n=69; age, 12-14 years; 88% of south Asian origin). Mean (±SD) baseline serum 25(OH)D concentration increased significantly in the intervention group (18.1±8.0 to 56±8.9 nmol/L) but not in the control group (17.9±7.4 to 15.7±6.6 nmol/L). Efficiency of movement increased significantly (by 5%; p=0.02) in the intervention group. An interaction was also found between baseline serum 25(OH)D concentration and jump velocity in the intervention group (p=0.02) with greater change in those with lower concentrations. There were no improvements in muscle force or power.

Summary – Muscle strength and function (children, 4-8 y and adolescents)

270. One RCT reported a beneficial effect of vitamin D supplementation on muscle function in adolescent girls with mean baseline serum 25(OH)D concentration of 18 nmol/L.

Adults < 50 years

Bone health indices

IOM report

271. Women of reproductive age were only considered during pregnancy and lactation. No trial data were available and only 1 cohort study was considered; therefore no conclusions could be drawn.

Evidence considered since IOM

Intervention studies

272. A 1-year RCT (Islam *et al*, 2010) reported a beneficial effect (p < 0.001) of daily vitamin D supplementation either alone (10 µg/400 IU), with calcium (600 mg), or with calcium plus a multiple micronutrient supplement compared with placebo on femur BMD and BMC in premenopausal women in Bangladesh (n=200; age 16-36 y). Mean baseline serum 25(OH)D concentration was 36 (±10.7) nmol/L. After 1 year, significantly (p < 0.001) higher mean serum 25(OH)D concentrations were observed in the vitamin D, vitamin D + calcium, vitamin D + Ca + MMN supplemented groups (increase of 32.2 ±23.7, 32.4 ±24.3, 28.8 ±24.8 nmol/L respectively) but not in the placebo group (increase of 0.6 ±13.8 nmol/L). However, the results of this study should be interpreted with caution since it was conducted in low-income Bangladeshi women with multiple micronutrient deficiencies and the findings may not be applicable to healthy young women in the UK.

273. No data were identified on vitamin D and indices of bone health in young adult men.

Summary – Bone health indices (adults < 50 years)

274. One RCT reported a beneficial effect of vitamin D supplementation on femoral BMD and BMC in premenopausal women in Bangladesh. These findings may not be applicable to premenopausal women in the UK.

Muscle strength and function

IOM Report

275. Muscle strength and function in adults under 50 years was not considered.

Evidence considered since IOM report

Systematic review and meta-analysis

276. Tomlinson *et al* (2014) investigated the effect of vitamin D supplementation on muscle strength in adults (< 40 y) in a systematic review and meta-analysis of 6 RCTs and 1 controlled trial (n=310; mean age 24 y). Three studies also administered calcium; in 2 studies both control and vitamin D groups were required to take calcium; in the 3rd study, participants were randomised to receive placebo, calcium, vitamin D₃ or vitamin D₃ and calcium. Mean baseline serum 25(OH)D concentration of participants (reported in 5 studies) was 30.8 nmol/L. Overall, vitamin D supplementation significantly improved upper (p=0.005) and lower (p=0.04) limb muscle strength.

Cohort studies

277. No cohort studies could be identified.

Summary – muscle strength & function (adults < 50 years)

278. Evidence from a small meta-analysis of 7 intervention studies reported that vitamin D supplementation improves limb muscle strength in adults with mean baseline serum 25(OH)D concentration of around 30 nmol/L.

Stress fracture prevention

279. Stress fractures are caused by repetitive sub-maximal loading of bone which ultimately results in a decrease in bone's intrinsic ability to repair itself, leading to an accumulation of microdamage. Stress fractures are therefore considered to be reflective of poor bone health and are a common problem in the younger, physically active population including many athletic groups (e.g., long distance runners). They are also a significant problem in military forces in the UK, US and Europe; for example, in the UK military, the current prevalence of pelvic stress fractures is 8-10% and tibia stress fractures is 6-7%.

IOM report

280. Data on vitamin D and stress fracture prevention in the younger adult population were not reviewed. A study that reported a reduction in the incidence of stress fractures in Navy recruits supplemented with a vitamin D and calcium was cited (Lappe *et al*, 2008) but its generalisability to the general population was questioned. This study is considered further below.

Evidence considered

Intervention studies

281. Lappe *et al* (2008) investigated the effect of vitamin D (20 µg/800 IU) and calcium (2000 mg) supplementation vs placebo for 8 weeks on stress fracture incidence in female Navy recruits (n=5201; age, 17-35y). Based on an intention to treat analysis, the calcium and vitamin D supplemented group had a 20% lower incidence of stress fracture than the control group (5.3% vs. 6.6%; p < 0.0026). Per protocol analysis of recruits who completed the study (n=3700) reported a 21% lower incidence of fractures in the supplemented vs the control groups (6.8% vs. 8.6% respectively; p<0.02). Although the results indicate a protective effect of vitamin D, no information was available on baseline or final serum 25(OH)D concentrations and the data are confounded because the supplement also included calcium. History of

exercise was also significantly protective for fracture; participants who exercised ≥ 3 times/wk had a 30% lower risk of stress fracture than those who exercised less ($p=0.004$). Other studies of military recruits have also reported decreased risk of fracture associated with regular physical activity (Shaffer *et al*, 2005; Rauh *et al*, 2006).

Cohort studies

Military populations

282. A systematic review and meta-analysis of 8 observational studies (5 prospective cohort; 2 nested case control; 1 case-control) examined the association between serum 25(OH)D concentration and stress fractures in military personnel ($n=2634$; aged 18-30 y) (Dao *et al*, 2014). In the individual studies, mean/median serum 25(OH)D concentration ranged between 45 and 82 nmol/L in stress fracture cases and 52 and 109 nmol/L in controls. In the 3 case control studies which measured serum 25(OH)D concentration at time of stress fracture diagnosis, the pooled mean difference was significantly lower in stress fracture cases compared with controls (-5.6 nmol/L; 95% CI, -9.7 to -1.6; $p=0.007$). In the 5 prospective cohort studies which measured serum 25(OH)D concentration at baseline, the pooled mean difference was not significantly lower between stress fracture cases and controls (-6.6 nmol/L; 95% CI, -14.5 to 1.3; $p=0.1$).
283. A subsequent cohort study (Davey *et al*, 2015) which prospectively followed Royal Marine (RM) recruits ($n=1082$ males; age, 16-32 y) through a 32 week training programme reported that recruits with baseline serum 25(OH)D concentration < 50 nmol/L had a higher incidence of stress fracture than those with concentrations > 50 nmol/L (OR, 1.6; 95% CI, 1.0-2.6; $p=0.042$).
284. Positive associations between serum 25(OH)D concentration and stress fracture risk in observational studies could be confounded by previous exercise which has been found to be protective of stress fracture risk (Lappe *et al*, 2008). Higher serum 25(OH)D concentration could be a proxy for previous exercise since people who regularly exercise are likely to spend more time outdoors and, as a consequence, have greater UVB exposure.

Non-military populations

285. Data for an association between serum 25(OH)D concentration and stress fractures in younger non-military populations are mainly observational, sparse and inconsistent.

Summary – stress fractures (adults < 50 years)

286. One intervention study reported a beneficial effect of vitamin D supplementation on stress fracture incidence in female Navy recruits, however the data could be confounded because the supplement also included calcium.
287. Evidence from a systematic review and meta-analysis of 8 observational studies in military personnel found a positive association between higher serum 25(OH)D concentration and a lower risk of stress fractures; however, a higher serum 25(OH)D concentration might be a proxy for previous exercise which is also protective of fracture risk.
288. One subsequent prospective cohort study in military recruits found serum 25(OH)D concentration < 50 nmol/L was associated with an increased risk of stress fracture.
289. The evidence in younger, non-military population groups is sparse and inconsistent.

Adults 50 years and over

Bone health indices

IOM report

290. The IOM reported discordance between results from RCTs and the majority of observational studies. A total of 19 studies were considered: 1 out of 6 RCTs, 4 out of 7 cohort studies and all 6 case-control studies reported an association between serum 25(OH)D concentration and BMD/bone loss. The IOM concluded that there was fair evidence from observational studies to support an association between serum 25(OH)D concentrations and BMD or changes over time in BMD at the femoral neck but not at other sites. Serum 25(OH)D concentration below which bone loss at the hip was increased ranged from 30 to 80 nmol/L.

Evidence considered since IOM report

Systematic review

291. A systematic review and meta-analysis (Reid *et al*, 2014) examined the effects of vitamin D supplements on BMD. A total of 23 trials (mean duration 23.5 months; n=4082; mean age 59 y²⁶) with mainly white populations were included. Mean serum 25(OH)D concentration at baseline was < 30 nmol/L in 5 studies (n=1181), 30-50 nmol/L in 3 studies (n=610), 50-75 nmol/L in 11 studies (n=1860) and > 75 nmol/L in 1 study (n=187). Calcium supplements were administered to all groups in 12 studies. No significant effect of vitamin D supplementation was found on BMD in either the spine or the total hip. There was a significant increase in femoral neck BMD (weighted mean difference 0.8%; 95% CI, 0.2-1.4; p=0.005) but there was evidence of heterogeneity in the data ($I^2=67%$; p<0.0003). The authors suggest this effect could be artifactual or a chance finding. Subgroup analysis showed that benefits were more pronounced in studies with vitamin D doses less than 20 µg/d (800 IU) in the lumbar spine; age, study duration or administration of calcium did not affect outcomes.

Intervention studies

292. A 3-year randomised population based open trial in Finland (Karkkainen *et al*, 2010) examined whether daily vitamin D (20 µg/800 IU) and Ca (1000 mg) supplementation could reduce bone loss in postmenopausal women (n=593; aged 65-71 y). The control group received no intervention. Mean (SD) serum 25(OH)D concentration at baseline was 50.1 (18.8) and 49.2 (17.7) nmol/L in the intervention and control groups respectively and 74.6 (21.9) nmol/L and 55.9 (21.8) nmol/L (p<0.001) respectively at the end of the trial. Total body BMD was significantly greater in the intervention group than in the control group (0.84% vs 0.19%; p=0.011) and BMD decrease at Ward's triangle was lower in the intervention group (-2.69% vs -2.83%; p=0.003). There were no differences between groups in BMD changes at the spine, femoral neck, trochanter and total proximal femur. Analyses in compliant women²⁷ showed significantly lower bone loss in femoral neck (-1.26% vs -1.73%, p=0.002), Ward's triangle (-1.63% vs -2.83%, p<0.0001), trochanter (0.25% vs -0.88%, p=0.001), and total proximal femur (-0.84% vs -1.47%, p<0.0001) compared to the control group. Total body BMD also increased more in the intervention group (+1.31% vs +0.19%, p=0.002). Bone loss at the lumbar spine, however, was greater in the intervention group (+0.67% vs +0.76%, p=0.03).

293. A 1-year RCT in Scotland (Macdonald *et al*, 2013) compared the effect of 2 different doses of vitamin D₃ supplementation (10 µg/400 IU or 25 µg/1000 IU) and placebo on BMD in postmenopausal women (n=305; mean age, 64.6 y). Mean BMD loss at the hip was significantly less in the group assigned to 25 µg

²⁶ Average age was < 50 y in 6 studies (n=871).

²⁷ Those who took at least 80% of their supplementation.

(1000 IU) of vitamin D₃ (p < 0.05) compared with the groups assigned to 10 µg (400 IU) vitamin D₃ or placebo. There were no differences in markers of bone metabolism (P1NP, CTX).

Cohort studies

294. A prospective study in 6 US centres with 4 years follow-up (Ensrud *et al*, 2009) reported an inverse association ($p_{\text{trend}} = 0.01$) between baseline serum 25(OH)D concentration and BMD loss rates (hip and trochanter) in community dwelling men (n=1279; age ≥ 65 y). The effect was observed mainly among men in the lowest quintile of baseline serum 25(OH)D concentration (< 47.7 nmol/L) where rate of hip bone loss was 1.5-fold higher than those in quintiles 2-5 (p=0.003). Hip bone loss rates were similar among men in the higher quintiles and not significantly different from each other. Subgroup analysis showed that lower serum 25(OH)D concentration was associated with higher rates of hip bone loss in men aged ≥ 75 y compared to < 75 y. No association was found between serum 25(OH)D concentration and rate of hip bone loss among men < 75 y.

Summary – bone health indices (adults > 50 years)

295. A systematic review and meta-analysis of 23 intervention trials reported a small benefit of vitamin D supplementation on femoral neck BMD (weighted mean difference 0.8%; 95% CI, 0.2-1.4) but no effect on BMD in either the spine or total hip.
296. Out of 2 RCTs not included in the systematic review, 1 found beneficial effects of supplementation on total body BMD and total hip BMD but not at other sites, while the other reported significantly less mean BMD loss at the hip with vitamin D supplementation.
297. One cohort study showed an association between serum 25(OH)D concentration < 50 nmol/L and greater rate of loss in hip BMD.

Fracture prevention

IOM report

298. The IOM reported that associations between serum 25(OH)D concentration and risk of fractures were inconsistent in the age group 51-70 y. For the age group ≥71 y, it concluded that supplementation with vitamin D₂ or D₃ did not reduce the risk of fractures but vitamin D (mainly vitamin D₃) plus calcium had a beneficial effect in reducing fractures in institutionalised older populations while the benefit in community dwelling individuals was inconsistent.

Evidence published since IOM report

Meta-analysis

299. A meta-analysis on the efficacy of oral vitamin D supplements in preventing non-vertebral and hip fractures among adults > 65 y included data from 12 RCTs (n=42,279) on non-vertebral fractures and 8 RCTs (n=40,886) on hip fractures (Bischoff-Ferrari *et al*, 2009). The pooled relative risk was 0.86 (95% CI, 0.77-0.96) for prevention of non-vertebral fractures and 0.91 (95% CI, 0.78-1.05) for prevention of hip fractures but there was significant heterogeneity for both end points. The pooled relative risk of trials which administered doses above 10 µg/d (400 IU) was 0.80 (95% CI, 0.72-0.89; 9 trials; n=33,265) for non-vertebral fractures and 0.82 (95% CI, 0.69-0.97; 5 trials; n=31 872) for hip fractures. The higher dose reduced non-vertebral fractures in community dwelling (29%) and institutionalised individuals (15%).

300. Bolland *et al* (2014) conducted a trial sequential meta-analysis²⁸ on the effect of vitamin D supplementation (alone and with calcium) on skeletal outcomes (total fracture & hip fracture; 22 trials; n=76,497; mean age 53-89 y), using a risk reduction threshold of 15%. There was statistically significant heterogeneity between the results of trials of vitamin D and trials of vitamin D plus calcium for hip fracture (p=0.004) but not for total fracture (p=0.4). Vitamin D alone did not reduce hip fracture by 15% or more (12 trials; n=27,834). Vitamin D plus calcium reduced hip fracture in institutionalised individuals (2 trials; n=3,853) but did not reduce the risk of hip fracture by 15% or more in community-dwelling individuals (7 trials; n=46,237).
301. A Cochrane review (Avenell *et al*, 2014) of 53 trials (n=91,791; mean/median age > 65 y) examined the effect of vitamin D and its analogues (1,25(OH)₂D) on fracture prevention. Vitamin D alone (in the forms and doses tested) vs placebo or no treatment had no effect on: hip fracture (11 trials; n=27,693; RR, 1.12; 95% CI, 0.98-1.29); non-vertebral fractures (12 trials; n=22,930; RR, 1.05; 95% CI, 0.96-1.14); vertebral fractures (6 trials, n=11,396; RR, 1.03; 95% CI, 0.76-1.39); or any new fracture (15 trials; n=28,271; RR, 1.03; 95% CI, 0.96-1.11). Vitamin D plus calcium was no more effective than calcium alone for: hip fracture (7 trials, n=7411; RR, 0.84; 95% CI, 0.63-1.13); any non-vertebral fracture (6 trials, n=3336; RR, 0.96; 95% CI, 0.76-1.16); and vertebral fracture (2 trials, n=2681; RR, 0.14; 95% CI, 0.01-2.77). Vitamin D plus calcium vs placebo or no treatment resulted in a statistically significant reduction in: risk of hip fracture (9 trials; n=49,853; RR, 0.84; 95% CI, 0.74-0.96); incidence of new non-vertebral fractures (8 trials; n=10,380; RR, 0.86; 95% CI, 0.78-0.96); incidence of any fracture (10 trials, n=49,976; RR, 0.95; 95% CI, 0.9-0.99). There was evidence of a statistically significant preventive effect of vitamin D plus calcium vs placebo or no treatment on clinical vertebral fractures (4 trials, n=42,185; RR, 0.89; 95% CI, 0.74-1.09).

Intervention studies

302. Sanders *et al* (2012) examined the effect of a single high annual dose of vitamin D₃ (12,500 µg/500,000 IU) for 3-5 years on fracture reduction in community dwelling women in Australia (n=2256; aged ≥ 70 y) and reported an increased risk of fractures in the vitamin D supplemented group compared to the placebo group (RR, 1.26; 95% CI, 1.00-1.59; p=0.047). Risk of falls was also increased in the vitamin D supplemented group (see paragraph 338).

Cohort studies

303. A nested case-control study (Cauley *et al*, 2010) within a prospective cohort study in the US examined associations between serum 25(OH)D concentration and fracture risk in men aged ≥ 65 y followed over an average of 5 years. Men with incident non-spine fractures (n=436) including hip fractures (n=81) were compared with a subcohort (n=1,608). One SD decrease in total serum 25(OH)D concentration was associated with an increased risk of hip fracture (multivariate HR=1.60; 95% CI, 1.18-2.17). Compared with men in the top quartile of serum 25(OH)D concentration (≥ 70 nmol/L), men in the lowest quartile (< 50 nmol/L) were at increased risk of hip fracture (HR, 2.36; 95% CI, 1.08-5.15; p trend=0.009). However, the association was attenuated by more than 50% (p trend=0.065) after adjustment for BMD. Serum 25(OH)D concentration was unrelated to non-spine fractures.
304. Cauley *et al* (2011) reported divergent associations between serum 25(OH)D concentration and risk of fracture in a cohort of multi-ethnic women (white, n=400; black, n=381; Hispanic, n=193; Asian, n=113; American Indian, n=46) followed over an average of 8.6 years. In multivariable models, serum 25(OH)D concentration > 50 nmol/L was associated with a lower risk of fracture in white women but a higher fracture risk in black women. Serum 25(OH)D concentration > 75 nmol/L was associated with higher

²⁸ Trial sequential analysis performs a cumulative meta-analysis but reduces the risk of false positive results from repetitive statistical testing by maintaining the overall risk of type 1 error at 5%.

fracture risk in Asian women; no significant associations were identified in the Hispanic or Native American women.

305. A cohort study in Japan (Nakamura *et al*, 2011) of community dwelling women (n=773; age, ≥ 69 y) followed up for 6 years, reported that the adjusted hazard ratios (HR) of limb and vertebral fractures for the first (< 48 nmol/L) and third quartile (59-71 nmol/L) of serum 25(OH)D concentration compared to the fourth quartile (≥ 71.0 nmol/L) were 2.82 (95% CI, 1.09-7.34) and 2.82 (95% CI, 1.09-7.27) respectively. However, the HR for the second quartile of serum 25(OH)D concentration (≥ 47.7 to < 59.2 nmol/L) compared to the fourth quartile was not significant (HR, 1.84; 95% CI, 0.68-4.98). The pooled adjusted HR was 0.42 (95% CI, 0.18-0.99) when the incidence in the fourth quartile (≥ 71.0 nmol/L) was compared to other three quartiles combined (< 71.0 nmol/L).
306. A cohort study in the USA, which followed community dwelling white and black participants (n=2614; age, > 70 y) for 6 years, found no evidence of an association between serum 25(OH)D concentration and hip and non-vertebral fractures (Barbour *et al*, 2012).
307. A cohort study (Rouzi *et al* 2012) which followed healthy postmenopausal women in Saudi Arabia (n=912; mean age, 61 y) for 5 years, reported that compared to being in the highest quartile of serum 25(OH)D concentration (45.1 nmol/L) being in the lowest quartile (≤ 17.9 nmol/L) was an independent risk factor for osteoporosis related fractures (RR, 1.63; 95%CI, 1.06-2.51).

Summary – fracture prevention (adults > 50 years)

308. Evidence from 3 meta-analyses on vitamin D supplementation and fracture prevention is mixed. One meta-analysis is supportive of a beneficial effect of vitamin D supplementation in reducing the risk of non-vertebral and hip fractures. In the 2 other meta-analyses, vitamin D alone had no effect on fracture risk but both meta-analyses suggested a beneficial effect of vitamin D plus calcium on fracture prevention.
309. One RCT reported an increased risk of fracture with a single high annual dose of vitamin D (12,500 μ g/500,000 IU).
310. Evidence from 5 cohort studies is mixed: 3 studies reported that serum 25(OH)D concentrations < 45 , < 50 and < 71 nmol/L are associated with increased fracture risk at some skeletal sites; 1 study found that a mean serum 25(OH)D concentration > 50 nmol/L is associated with lower fracture risk in white women but a higher fracture risk in black women; 1 study found no association between serum 25(OH)D concentration and fracture risk.

Muscle strength and function

IOM Report

311. Although the IOM considered ‘*physical performance*’ and ‘*falls*’ as independent indicators the evidence for both was considered together because of the integration of these indicators in the literature reviewed. The IOM concluded that there was a lack of sufficiently strong evidence (from RCTs and observational associations) on vitamin D with or without calcium and risk of falls and poor physical performance to support DRI development. Evidence from RCTs in particular showed outcomes that varied in significance and did not support observational findings or a causal relationship. For physical performance the IOM concluded that, overall, data from RCTs suggested that vitamin D doses of at least 20 μ g/d (800 IU), either alone or in combination with calcium, may have beneficial effects on measures of physical performance; however, the evidence was considered insufficient to define the shape of the dose-response curve for higher intakes.

Evidence considered since IOM report

Systematic reviews & meta-analyses

312. A systematic review and meta-analysis of 13 RCTs (n=2,268; mean age 78±4 y) assessed the efficacy of vitamin D supplementation on muscle strength, gait and balance (Muir *et al*, 2011). A significant improvement was reported in postural sway (p=0.04), timed up and go test (p=0.03) and lower extremity muscle strength (p=0.04) but not on gait. Mean serum concentrations (provided in 12 studies) ranged between 24.5 and 65.7 nmol/L at baseline (and was < 50 nmol/L in 10 out of 12 studies).
313. Stockton *et al* (2011) conducted a meta-analysis of 17 RCTs (n=5,072). Participants were aged > 60 years in most studies but 2 studies included younger adults (50-79 y & 31.6±4.8). No significant effect of vitamin D supplementation was found on grip strength or proximal lower limb strength when mean baseline serum 25(OH)D concentrations were > 25 nmol/L; pooled data from two studies in which the mean baseline serum 25(OH)D concentration was < 25 nmol/L reported a significant improvement in hip muscle strength (SMD 3.52, 95% CI 2.18, 4.85). However, both studies were conducted in chronically hospitalised patients in Japan: 1 in stroke patients with hemiplegia (Sato *et al*, 2005a) and the other in patients with Alzheimer's disease (Sato *et al*, 2005b). The vitamin D intervention in the study with Alzheimer's patients was 15 minutes of sunshine exposure every day. Out of the 2 other RCTs with mean baseline serum 25(OH)D concentration < 25 nmol/L, 1 (Gupta *et al*, 2010) was of younger participants (mean age 31.6 ± 4.8 y) and reported a statistically significant difference between treatment and control groups in grip (p<0.001) and calf (p=0.04) strength but not in pinch grip strength (p=0.07); the other RCT (Corless *et al*, 1985) as with hospitalised patients and found no significant effect of vitamin D supplements on strength score (derived from functional activities).
314. Another systematic review and meta-analysis (Beaudart *et al*, 2014) included 30 RCTs (n=5,615; mean age 61 y). Supplementation was with vitamin D alone in 14 studies and combined vitamin D and calcium in 16 studies; 4 studies used vitamin D analogues (alfacalcidol and 1,25(OH)₂D). A small but significant positive effect of vitamin D supplementation on global muscle strength was reported (SMD, 0.17; 95% CI, 0.03-0.31; p=0.02) but there was significant heterogeneity (p< 0.001; I², 77.7%). The improvement in muscle strength was significantly greater when mean baseline serum 25(OH)D concentration was < 30 nmol/L (p=0.02), in people aged ≥ 65 years (SMD 0.25; 95% CI, 0.01-0.48) and in hospitalised people compared to community dwellers (p<0.01). There was no significant effect of vitamin D supplementation on muscle mass or power.

Intervention studies

315. Lips *et al* (2010) compared the effect of a weekly vitamin D₃ supplement (210 µg/8,400 IU) or placebo for 16 weeks on postural stability and muscle strength in adults ≥ 70 y in the US and Europe (n=226). Mean serum 25(OH)D concentration increased from 34.7 to 65.5 nmol/L with supplementation but there was no significant difference in postural sway or short physical performance battery (SPPB) scores between the treatment and placebo groups. A *post hoc* analysis of participants subgrouped by postural sway at baseline (≥ 0.46 compared with <0.46 cm) showed an improvement in postural sway with vitamin D supplementation compared with placebo (p=0.047).
316. Knutsen *et al* (2014) compared the effect of daily vitamin D₃ supplementation (either 10 µg/400 IU or 25 µg/1000 IU) or placebo for 16 weeks on muscle strength and power in adults from minority ethnic groups (n=251; aged 18-50 y) living in Norway. The main outcome measure was the difference in jump height pre and post intervention; secondary outcomes were differences in handgrip strength and chair-rising test. Mean baseline serum 25(OH)D concentration was 27 nmol/L (range, 5-87 nmol/L) which increased to 43 nmol/L in the group supplemented with 10 µg/d (400 IU) vitamin D and to 52 nmol/L in the group supplemented with 25 µg/d (1,000 IU). Vitamin D supplementation had no significant effect on jump height, handgrip strength or chair-rising test in any group.

317. A small RCT in Australia (n=26; age, ≥ 60 y; baseline serum 25(OH)D concentration, 25-60 nmol/L) with the primary aim of assessing the effect of 50 $\mu\text{g}/\text{d}$ (2000 IU) of vitamin D₃ for 10 weeks on neuroplasticity, also measured muscle strength and function (Pirodda *et al*, 2014). Mean serum 25(OH)D concentration increased from 46 to 81 nmol/L in the vitamin D treated group, with no change in the placebo group. There was a significant 8-11 % increase ($p < 0.05$) in muscle strength in the vitamin D supplemented group but the changes were not significantly different from the placebo group. Vitamin D supplementation had no effect on muscle power. However, this study was limited by the small sample size and relatively short duration.

Cohort studies

318. Scott *et al* (2010) examined the association between baseline serum 25(OH)D concentration and muscle function in community dwelling adults in Tasmania (n=686; mean age 62 ± 2 y; 49% women) followed for 2.6 years. At baseline, participants with a serum 25(OH)D concentration ≤ 50 nmol/L had significantly lower appendicular lean mass, leg strength, leg muscle quality and physical activity (all $p < 0.05$). After adjustment for potential confounders, baseline serum 25(OH)D concentration was an independent predictor of change in leg strength over time ($p = 0.027$).
319. Another prospective study (Menant *et al*, 2012) of community dwelling adults in Australia (n=463; aged 70-90 years) reported that participants with serum 25(OH)D concentration ≤ 50 nmol/L had weaker upper and lower limb strength, poorer balance and slower gait speed. Men (but not women) with serum 25(OH)D concentration ≤ 50 nmol/L also had a significantly higher risk of falls during the 12 months follow up (IRR=1.94; 95% CI, 1.19–3.15; $p = 0.008$).
320. A longitudinal analysis in the US (North Carolina) of community dwelling people (n=988; age, 77-100 y) (Houston *et al*, 2011) with 3 years follow-up reported that SPPB scores and grip strength were lower in participants with serum 25(OH)D concentration < 50 nmol/L compared to > 75 nmol/L after adjustment for confounding factors. During 3 years follow up, participants with serum 25(OH)D concentration < 50 nmol/L were at greater risk of impaired mobility (HR=1.56; 95% CI, 1.06-2.30).
321. A longitudinal study in Australia (Bolland *et al*, 2010) examined the association between serum 25(OH)D concentration and multiple health outcomes in community dwelling women (n=1,471; mean age, 74 y) followed up in a 5 year trial of calcium supplementation. Seasonally adjusted serum 25(OH)D concentration at baseline was < 50 nmol/L in 50% of the women. There was no increase in risk of adverse consequences for any musculoskeletal outcome including loss of grip strength or falls after adjustment for comorbidities and other confounding factors.
322. Another prospective study in Hong Kong followed community dwelling men (n=714; age ≥ 65 y) over 4 years (Chan *et al*, 2012) and reported that $> 90\%$ had a serum 25(OH)D concentration ≥ 50 nmol/L at baseline. After adjustment for potential confounding factors, serum 25(OH)D concentration was not associated with baseline or change in appendicular skeletal muscle mass or physical performance measures including grip strength, chair standing time or walking speed.
323. Michael *et al* (2011) assessed measures of muscle strength in postmenopausal women (n=523) taking part in the Women's Health initiative Clinical Trial (USA). A physical performance summary score was derived from three tests: timed walk, chair-stand, and grip strength. Mean (\pm SD) baseline serum 25(OH)D concentration was 48.2 (± 21.4) nmol/L. Across 6 years of follow-up, participants with baseline serum 25(OH)D concentration ≥ 75 nmol/L had higher physical performance scores compared to those with serum 25(OH)D concentration < 35 nmol/L (p trend 0.01). Baseline 25(OH)D concentration had no effect on rate of decline in physical performance.

324. Houston *et al* (2012) examined longitudinal associations between serum 25(OH)D concentration and physical performance and strength in a cohort of men and women in the US (n=2,641; age, 71–80 y). Serum 25(OH)D concentration and physical performance and strength were measured at baseline and after 2 and 4 years. Compared to participants with serum 25(OH)D concentration ≥ 75 nmol/L, a concentration < 50 nmol/L was associated with poorer physical performance at 2 and 4 years ($p < 0.01$). Although physical performance and strength declined over 4 years ($p < 0.0001$), rate of decline was not associated with baseline serum 25(OH)D concentration.

Summary – muscle strength and function (adults > 50 years)

325. Three meta-analyses of RCTs reported a beneficial effect of vitamin D supplementation on muscle strength and function in adults > 50 years with mean baseline serum 25(OH)D concentration of 24-66 nmol/L, < 30 nmol/L and < 25 nmol/L; however the latter was based on 2 studies in hospitalised patients in Japan and may not be applicable to the general population in the UK.
326. Three subsequent RCTs were largely unresponsive of an effect of vitamin D supplementation on muscle strength.
327. Out of 7 cohort studies, 5 found an association between serum 25(OH)D concentration and muscle strength and function in people with baseline serum 25(OH)D concentration < 50 nmol/L; however, cut-offs were predefined in most studies.

Falls

328. In studies that have examined the relationship between vitamin D and fall risk, the outcomes usually considered are '*risk of falling*' and '*risk of being a faller*'. Being a faller is a yes/no response & does not take into consideration the fact that some fallers might fall once while others might fall on multiple occasions. Since any fall might lead to a fracture, stopping people from falling at all would be the best outcome; i.e., converting someone from being a faller to a non-faller. It is easier to demonstrate a reduction in falls but, from a public health perspective, a decrease in the risk of being a faller is probably more important. Among falls experts, the latter is generally regarded as a better marker of efficacy of an intervention. Both outcomes are considered in the studies below.

IOM report

329. The IOM report considered '*falls*' and '*physical performance*' together because of the integration of these indicators in the literature reviewed. It concluded that there was a lack of sufficiently strong evidence (from RCTs and observational associations) on vitamin D with or without calcium and risk of falls and poor physical performance to support DRI development. Evidence from RCTs in particular showed outcomes that varied in significance and did not support observational findings or a causal relationship. The evidence was also not constantly supportive for a role of vitamin D combined with calcium in reduction of risk for falls.
330. The IOM considered 2 meta-analyses by Bischoff-Ferrari *et al* (2004; 2009). The 2004 meta-analysis (5 RCTs; n=1,237) reported that vitamin D supplementation reduced the risk of falling by 22% (corrected OR, 0.78; 95% CI, 0.64-0.92) compared with calcium or placebo. However, 2 of the included RCTs administered activated metabolites of vitamin D (either 1,25(OH)2D or 1 α -hydroxycholecalciferol) rather than vitamin D itself and the study which used 1,25(OH)2D was the only trial that reported a significant reduction in falls. Mean serum 25(OH)D concentration ranged from 25.7-78 nmol/L at baseline to 40.5-65²⁹ nmol/L after supplementation. A secondary analysis which included 5 additional studies (n=1,001) in a sensitivity analysis reported a smaller but still significant effect size (corrected RR=0.87, 95% CI, 0.80-0.96).

²⁹ Concentrations declined because intervention was active D.

331. The 2009 meta-analysis included 8 RCTs (n=2,426) in the primary analysis but analysed separately the 2 trials which uses an activated metabolite of vitamin D. Overall, there was a borderline reduction in fall risk with vitamin D supplementation (pooled RR=0.87, 95% CI, 0.77-0.99) but there was heterogeneity among trials for dose of vitamin D and achieved serum 25(OH)D concentration. Vitamin D doses of 17.5-25 µg/d (700-1,000 IU) reduced fall risk (pooled RR 0.81, 95% CI, 0.71-0.92) but doses of 5-10 µg/d (200-400 IU) did not (pooled RR=1.10, 95% CI, 0.89-1.35). Fall risk was also reduced with achieved serum 25(OH)D concentrations ≥ 60 nmol/L (pooled RR=0.77, 95% CI, 0.65-0.90) but not with concentrations < 60 nmol/L (pooled RR 1.35, 95% CI, 0.98-1.84). Active forms of vitamin D reduced fall risk by 22% (pooled RR 0.78, 95% CI 0.64-0.94).
332. The IOM report highlighted a number of limitations in these meta-analyses which may have influenced the overall results, including omission of some studies that met the inclusion/exclusion and inclusion of 1 study that failed to meet the inclusion criteria. Another criticism related to the inappropriate presentation and interpretation of the meta-regression analysis of the relative risk against vitamin D dose or achieved serum 25(OH)D concentration. A reanalysis of the meta-analysis by the IOM reported a null effect of vitamin D supplementation on falls.

Evidence considered since IOM report

Systematic reviews and meta-analyses

333. A Cochrane Review (Cameron *et al*, 2012) which assessed the effect of vitamin D supplementation on fall prevention in adults aged > 65 y³⁰ in nursing care facilities and hospitals (5 trials; n=4603) reported a significant reduction in rate of falls (rate ratio=0.63, 95% CI, 0.46-0.86; 5 trials; n=4603) but not risk of falling (RR, 0.98, 95% CI 0.89 to 1.09; 6 trials³¹; n=5186). Mean baseline serum 25(OH)D concentrations in the included studies ranged from 23 to 59 nmol/L.
334. A second Cochrane Review investigated the effect of vitamin D supplementation on fall prevention in community dwelling adults aged ≥ 60 years³² (Gillespie *et al*, 2012). Overall, vitamin D did not reduce rate of falls (RaR³³, 1.00; 95% CI, 0.90-1.11; 7 trials; n=9324) or risk of falling (RR, 0.96; 95% CI, 0.89-1.03; 13 trials; n=26,747). A *post hoc* subgroup analysis of 4 trials that specifically recruited participants with 'low' baseline serum 25(OH)D concentration³⁴ reported a greater reduction in rate of falls (RaR, 0.57; 95% CI, 0.37-0.89; 2 trials³⁵; n=260; baseline serum 25(OH)D concentration, 24-28 nmol/L) and risk of falling (RR, 0.70; 95% CI, 0.56-0.87; 4 trials³⁶; n=804; baseline serum 25(OH)D concentration, 24-55 nmol/L). For trials that did not select on baseline serum 25(OH)D concentration, vitamin D supplementation had no effect on rate of falls (RaR, 1.02; 95% CI, 0.88-1.19; 3 trials; n=3669) or risk of falling (RR, 1.00; 95% CI, 0.93-1.07; 9 trials; n=25,943).
335. A meta-analysis (Kalyani *et al*, 2010) of 10 RCTs in community dwelling and institutionalised adults ≥ 60 years (n=2,932) reported a significant reduction in falls with supplementation (RR, 0.86; 95% CI, 0.79-0.93), with fewer falls in the following subgroups: those aged < 80 y; when calcium was co-administered, when vitamin D₃ was used at doses > 20 µg/d (800 IU), supplementation > 6 months and there was no previous history of falls or fractures. Mean baseline serum 25(OH)D concentrations in the included studies ranged from 23 to 82 nmol/L. Meta-regression analysis showed no significant linear association between vitamin D dose or duration and risk of falls.

³⁰ Trials were also included if the mean age was > 65 years.

³¹ 1 trial tested a vitamin D supplement that included vitamin D plus calcium (Grieger, 2009).

³² Trials also included if mean age minus 1 standard deviation was more than 60 years.

³³ Rate ratio.

³⁴ Baseline serum 25(OH)D in the 4 trials with lower concentrations: range 23.7-28 nmol/L (Dhesi *et al*, 2004), mean (SD) 25.2±12.9 nmol/L (Pfeifer *et al*, 2000), mean (SD) 54.5±18 nmol/L (Pfeifer *et al*, 2009); mean (SD) 44.8±12.7 nmol/L (Prince *et al*, 2008).

³⁵ Dhesi *et al* (2004); Pfeifer *et al* (2000).

³⁶ Dhesi *et al* (2004); Pfeifer *et al* (2000); Pfeifer *et al* (2009); Prince *et al* (2008).

336. Another meta-analysis (Murad *et al*, 2011) of 26 trials (n=45,782; mean age 76 y) reported vitamin D supplementation significantly reduced fall risk (OR of at least 1 fall, 0.86; 95% CI, 0.77-0.96) but noted substantial heterogeneity across studies ($I^2=66\%$). Sub-group analysis showed risk reduction was greater in participants who were considered vitamin D 'deficient'³⁷ at baseline but the mean serum 25(OH)D concentration in this subgroup was not specified. Risk reduction was also greater when calcium was co-administered (vitamin D + calcium vs placebo, 10 trials, OR=0.83; 95% CI=0.72-0.93; vitamin D + calcium vs calcium, 10 trials, OR=0.63; 95% CI, 0.50-0.81). Vitamin D alone vs placebo did not reduce risk of fall reduction (OR=0.97; 95% CI, 0.84-1.11). The authors concluded that vitamin D with calcium reduces the risk of falls however publication bias had likely affected the results and exaggerated the estimates of risk reduction.
337. A meta-analysis of 20 trials (n=29,535; mean age range, 71-89 y) (Bolland *et al*, 2014) reported no effect of vitamin D supplementation, with or without calcium, on risk of falls (RR, 0.96; 95% CI, 0.91-1.01). Subgroup analyses did not find significant interactions between baseline or achieved serum 25(OH)D concentration and fall risk. Sixteen trials reported serum 25(OH)D concentration (mean range, 22 to 75 nmol) with baseline serum concentration < 50 nmol/L in 12 trials. All trials reported increases in serum 25(OH)D concentration and 14 trials reported that serum 25(OH)D concentration increased to > 50 nmol/L in the vitamin D intervention group. The need for further randomised trials on effects of vitamin D supplements on falls was assessed using trial sequential analysis³⁸ with a risk reduction threshold of 15%. In the 20 RCTs included in the analysis, the effect estimate for vitamin D supplementation (with or without calcium) on falls lay within the futility boundary, suggesting that vitamin D supplementation does not alter the relative risk of falls by 15% or more.

Intervention studies

338. An RCT of community-dwelling women (n=2,256; age ≥ 70 y) randomised to receive a vitamin D supplement (12,500 µg/500,000 IU) or placebo on an annual basis for 3-5 years (Sanders *et al*, 2010) reported a significant increase in rate of falls (RaR 1.15; 95% CI, 1.02-1.30) and fractures (RR 1.26, 95% CI 1.00-1.59) in the vitamin D supplemented group compared to placebo. A *post hoc* analysis indicated that the excess falls and fractures occurred in the 3 months after dosing, when median serum 25(OH)D concentration was approximately 120 nmol/L after 1 month and 90 nmol/L after 3 months. This RCT used very high supplementation doses (provided as annual bolus) which may elicit different effects from daily supplementation.

Cohort studies

339. Menant *et al* (2012) assessed the relationship between serum 25(OH)D concentration and falls in a cohort of community dwelling adults (n=463; age, 70-90 years) followed for 1 year. Rate of falls was significantly increased in men with serum 25(OH)D concentration < 50 nmol/L (IRR=1.94; 95% CI, 1.19-3.15; p=0.008) but not in women.

Genetic studies

340. A study in Italian adults (n=259; mean age 85±4.5y; 66% women) reported that the bb genotype of the *Bsm1* VDR gene was associated with a reduced risk of falls compared with the BB genotype (Onder *et al*, 2008). A subsequent study examined VDR polymorphism and falls risk in two separate cohorts (Barr *et al*, 2010), the Aberdeen Prospective Osteoporosis Screening Study (APOSS; n=3,209) and the Osteoporosis

³⁷ Studies categorised as having a vitamin D-deficient vs not deficient population based on: author description; baseline serum 25(OH)D concentration or inclusion of participants with at least 2 vitamin D deficiency risk factors (old age, dark skin, living in a nursing home, living far from the equator, winter season, sunscreen use, wearing a veil, smoking, obesity, malabsorption disease, renal or liver disease, and use of medication).

³⁸ Trial sequential analysis permits estimation of the point at which the evidence base is large and consistent enough to make further trials futile because of the low probability that they will affect results of existing meta-analyses (Wetterslev *et al*, 2008).

and Ultrasound Study (OPUS; n=1,970) and found an increase in falls risk with the BB *Bsm1* genotype in both cohorts. An association was also found between *Bsm1* polymorphism and balance and muscle power measurements. There was no association between risk of falls and serum 25(OH)D concentration.

Summary – Falls (adults > 50 years)

341. Evidence from meta-analyses of RCTs on vitamin D and falls is mixed. Out of 5 meta-analyses, 3 reported some beneficial effect of vitamin D supplementation on reducing the rate of falls and/or risk of falling in adults > 50 years with mean baseline 25(OH)D concentrations ranging between 23 and 59 nmol/L, 24 and 28 nmol/L, 24 and 55 nmol/L and 23 and 82 nmol/L; 1 meta-analysis reported a beneficial effect of vitamin D supplementation only when it is administered with calcium; 1 meta-analysis found no beneficial effect of vitamin D supplementation with or without calcium on risk of falls.
342. One RCT reported that a single high annual dose of vitamin D (12,500 µg/500,000 IU) increases the risk of falls.
343. One cohort study found an association between a mean serum 25(OH)D concentration < 50 nmol/L and increased rate of falls in men but not in women.
344. One genetic study reported that the bb genotype of the *Bsm1* VDR gene was associated with reduced fall risk compared with the BB genotype. A subsequent study found that the BB genotype was associated with increased fall risk.

Conclusions - vitamin D and musculoskeletal outcomes

Rickets

345. Evidence on rickets in infants and children is mainly observational and therefore has the potential for confounding. In case reports, individual serum 25(OH)D concentrations ranged between < 2.5 and < 50 nmol/L; in observational and intervention studies, mean/median concentrations ranged between 5 and 50 nmol/L. A clear threshold serum 25(OH)D concentration above which there is no risk of rickets could not be identified from the evidence considered; however, rickets was present at individual and mean serum 25(OH)D concentrations < 25 nmol/L (the current threshold for increased risk of rickets) in the majority of studies.
346. It is not known whether the rickets was caused solely by vitamin D deficiency in all of the studies considered since most did not provide information on calcium intakes. It is possible, therefore, that the presence of rickets at serum 25(OH)D concentrations above 25 nmol/L might be caused by calcium deficiency.
347. Although the risk of rickets is increased at serum 25(OH)D concentrations < 25 nmol/L, a concentration below this level is not diagnostic of the disease.

Osteomalacia

348. Evidence on vitamin D and osteomalacia in adults is limited and drawn mainly from case reports. It is not possible to discern a serum 25(OH)D concentration below which there is a clear increase in risk of osteomalacia. Serum 25(OH)D concentrations ranged between 4 and 20 nmol/L in case reports; out of 2 cross-sectional analyses, the mean serum 25(OH)D concentration was 15 nmol/L in one study and < 7.5 nmol/L for all participants in the other. A minimum serum 25(OH)D concentration associated with mineralisation defects could not be identified in a post-mortem analysis of bone biopsies.

Bone health indices beyond rickets and osteomalacia

349. Bone health indices (BMC/BMD/biochemical markers of bone formation and resorption) were considered across all life stage groups. Findings from studies that considered the effect of vitamin D supplementation

on bone health indices or associations between serum 25(OH)D concentration and bone health indices varied by life stage. Evidence was suggestive of a positive association between maternal 25(OH)D concentration during pregnancy and bone health indices in the fetus/newborn, however the physiological significance of this is not known. Evidence was also suggestive of beneficial effects of vitamin D supplementation on bone health indices at some skeletal sites in adults aged > 50 years. Effects of vitamin D supplementation on bone health indices of infants, children and adolescents is inconsistent but the majority of RCTs did not find any effect. The evidence base for children aged 1-3 years and adults < 50 years was too small to draw any conclusions.

Fracture prevention

350. Data on vitamin D supplementation and fracture prevention in adults > 50 years is mixed but suggests that vitamin D plus calcium is more effective in reducing fracture risk than vitamin D alone. On balance, vitamin D supplements have no beneficial effect on fracture risk in adults > 50 years.
351. Evidence on the effect of vitamin D supplementation or serum 25(OH)D concentration and stress fracture risk in younger age groups is insufficient to draw firm conclusions.

Muscle strength and function

352. Limited evidence suggests a beneficial effect of vitamin D supplementation on muscle function in adolescent girls with a mean serum 25(OH)D concentration < 18 nmol/L and in adults < 50 years with a mean serum 25(OH)D concentration < 30 nmol/L.
353. In adults > 50 years, evidence is mixed but, overall, is suggestive of a beneficial effect of vitamin D supplementation on muscle strength and function at mean baseline serum 25(OH)D concentrations ranging between < 25 and 66 nmol/L. Evidence from cohort studies is also supportive of an association between mean serum 25(OH)D concentration and muscle strength and function when baseline serum 25(OH)D concentration is < 50 nmol/L.

Falls

354. Evidence on vitamin D and falls is mixed but, overall, is suggestive of a beneficial effect of vitamin D supplementation in reducing fall risk in adults > 50 years with mean baseline serum 25(OH)D concentrations ranging between < 25 and around 80 nmol/L.
355. Although one study reported that vitamin D supplementation increased fall risk, the supplementation dose was very high (12,500 µg/500,000 IU) and was administered annually which may have different effects from daily supplementation.

Non-musculoskeletal health outcomes

(See Tables 28-47, Appendix 1)

Pregnancy and lactation: non-skeletal outcomes in mother and baby

356. Serum 25(OH)D concentration is stable or falls slightly during pregnancy and 1,25(OH)₂D concentration approximately doubles from the first trimester until delivery because the DBP rises (Kovacs, 2008).

Effect of vitamin D supplements during pregnancy on serum/plasma 25(OH)D concentration

357. A controlled trial (non-randomised, unblinded) trial in Edinburgh supplemented women (n=506) from the 12th week of pregnancy until delivery with 10 µg/d (400 IU) of vitamin D₂ (Cockburn *et al*, 1980). Mean maternal plasma 25(OH)D concentration at delivery was 43 nmol/L in the intervention group compared to 33 nmol/L in the control group. The distribution of values in both groups was described as highly skewed but the variance, or information on compliance, was not reported. Two RCTs in South Asian or women from black and minority ethnic groups (Brooke *et al*, 1981; Yu *et al*, 2009), 1 controlled study (Congdon *et al*, 1983) and 1 uncontrolled study (Datta *et al*, 2002) used vitamin D supplemental amounts of 20-25 µg/d (800-1000 IU). The RCTs demonstrated a significant increase in serum 25(OH)D concentration between baseline sample concentration and delivery but the effect size was highly discrepant. The plasma 25(OH)D concentrations reported by Brooke *et al* (1981) were almost double those measured in any other study and exceeded concentrations observed in American women with higher baseline concentrations consuming 100 µg/d (4000 IU) of vitamin D₃ daily at a more southerly latitude (S Carolina, US) (Hollis *et al*, 2011). This observation is very hard to explain.
358. Yu *et al* (2009) compared two methods of oral supplementation: 20 µg/d (800 IU) for 27 weeks vs a single amount of 5000 µg (200,000 IU) at 27 weeks gestation. Both interventions appeared equally effective overall as observed also in a study in Lyon (Delvin *et al*, 1986).

IOM Report

359. In relation to maternal non-skeletal outcomes, the IOM report considered preeclampsia/pregnancy induced hypertension (PIH). No RCTs were identified. Two observational studies reported associations between vitamin D and pre-eclampsia/PIH incidence but the data were not conclusive. In relation to the effect of maternal serum 25(OH)D concentration on newborn non-skeletal health outcomes it noted conflicting evidence from RCTs and observational studies.

Non-skeletal outcomes in the newborn

360. A key functional outcome of maternal serum 25(OH)D in pregnancy is provision of a fetal/infant vitamin D 'store' to meet later demands of the unsupplemented breastfed infant alongside endogenous synthesis and intake from milk. Where both maternal and infant serum 25(OH)D concentration at birth have been measured in UK studies, fetal cord plasma 25(OH)D concentration is around 60-70% of maternal value, slightly lower than the ratio cited in some reviews (68-108%) (Greer, 2008).

Neonatal hypocalcaemia

Intervention studies

361. Two UK controlled trials (Cockburn *et al*, 1980; Brooke *et al*, 1981) and a French study (Delvin *et al*, 1986) reported a reduction in neonatal hypocalcaemia incidence with maternal vitamin D supplementation in pregnancy.
362. The study by Cockburn *et al* (1980) was not randomised but study groups were treated concurrently (allocation by hospital ward). Participants (n=1139) received a vitamin D₂ supplement (10 µg/400 IU) or placebo daily from the 12th week of pregnancy until delivery. Umbilical venous blood was collected at

delivery and a capillary sample taken from the infant on the 6th day of life. Maternal plasma 25(OH)D concentration was measured at 24 and 35 weeks of pregnancy and at delivery. Mean maternal 25(OH)D concentration at delivery was 43 nmol/L in supplemented women and 33 nmol/L in controls. Corresponding 25(OH)D concentrations in umbilical venous blood were 28 and 20 nmol/L. Neonatal hypocalcaemia (defined as plasma $\text{Ca}^{2+} < 1.85 \text{ mmol/L}$) occurred in 6% of the intervention group infants and 13% of controls ($p < 0.005$). Birth weight and length were not measured. Out of 61 infants who had their teeth examined in their 3rd year, a significantly higher incidence of dental enamel hypoplasia was observed in those born to control mothers and who had been hypocalcaemic compared to those born to supplemented mothers.

363. Brooke *et al* (1981) randomly allocated women of South Asian ethnic origin ($n=126$) to receive vitamin D₂ (25 µg/1000 IU/d) or placebo in a double-blind design. Five control infants but no treatment group infants developed symptomatic hypocalcaemia (plasma $\text{Ca}^{2+} < 1.8 \text{ mmol/L}$). At delivery, between group differences in mean maternal plasma 25(OH)D concentration (168 nmol/L in intervention group and 16 nmol/L in control group) and mean umbilical venous plasma 25(OH)D concentration (138 nmol/L in intervention group and 10 nmol/L in control group) were very striking and have not been replicated in any subsequent studies.
364. Delvin *et al* (1986) randomly assigned pregnant women ($n=40$) in the 6th month of pregnancy to receive either vitamin D₃ (25 µg/1000 IU/d) or no treatment. There was a significant decrease ($p < 0.002$) in serum calcium at 4 days of age in both groups although to a lesser extent in infants born to the supplemented mothers ($p < 0.05$)

Observational studies

365. A prospective national population-based survey (Basatemur & Sutcliffe, 2015), which estimated the incidence of hypocalcaemic seizures due to vitamin D deficiency³⁹ in children (0-15 y; $n=91$) resident in the UK and Ireland, reported that serum 25(OH)D concentration was $< 25 \text{ nmol/L}$ in 86% of children who developed a hypocalcaemic seizure.

Birth weight and length, small for gestational age

Intervention studies

366. A systematic review (Harvey *et al*, 2014) identified 9 intervention studies ($n=40-350$). Only 1 study (Brooke *et al*, 1980) was double-blinded and placebo controlled. Three studies (all from India) reported significantly greater birth weight in infants of supplemented mothers. None of the 3 UK studies (Brooke *et al*, 1980; Congdon *et al*, 1983; Yu *et al*, 2009) reported significant differences in infant birth weight between supplemented and unsupplemented groups. The largest study included in the review (Hollis *et al*, 2011) was of pregnant women in the US ($n=500$; 12-16 weeks gestation) randomised⁴⁰ to receive 10 µg (400 IU), 50 µg (2000 IU) or 90 µg (3600 IU) of vitamin D₃/d. There were no differences between the groups in gestational age at delivery or birth weight. Meta-analysis of the studies did not find a significant difference in birth weight between supplemented and unsupplemented groups. Out of 2 RCTs which examined birth length, 1 (Brooke *et al*, 1980) found no effect of daily vitamin D₂ supplementation (25 µg/1000 IU) in the last trimester while the other (Marya *et al*, 1989) reported significantly higher birth length ($p < 0.001$) in women supplemented with vitamin D₃ (15,000 µg/600,000 IU in 7th & 8th month of gestation). Two trials (Brooke *et al*, 1980; Yu *et al*, 2009) evaluated the effect of vitamin D

³⁹ Paediatricians were asked to report a suspected seizure in the presence of both serum corrected calcium $< 2.0 \text{ mmol/L}$ and serum 25(OH)D $< 50 \text{ nmol/L}$.

⁴⁰ Randomisation was incomplete since some mothers allocated to the 50 µg/d (2000 IU) vitamin D₃ group were switched to other groups because of pre-existing high baseline serum 25(OH)D concentration ($> 100 \text{ nmol/L}$).

supplementation on risk of offspring being born small for gestational age⁴¹ (SGA). Both trials found no significant differences in SGA between supplemented and unsupplemented groups.

367. An RCT (Wagner *et al*, 2013a) not included in the Harvey *et al* (2014) review, of pregnant women (n=257; 12-16 weeks gestation) supplemented daily with vitamin D₃ (50 µg/2000 IU or 100 µg/4000 IU) reported no differences in birth weight, gestation or neonatal health. A combined analysis of the RCTs (n=759) by Wagner *et al* (2013a) and Hollis *et al* (2011) also found no differences in birth weight, gestation or neonatal health (Wagner *et al*, 2013b). Data on birth length were not provided.

Observational studies

368. The systematic review by Harvey *et al* (2014) included 19 observational studies linking maternal serum 25(OH)D concentration to infant birth weight (14 cohort, 5 cross-sectional); 3 out of 14 studies that had measured maternal serum 25(OH)D concentration reported a significant positive association with infant birth weight. Eight cohort studies examined associations between maternal serum 25(OH)D concentration and offspring birth length. No relationship was found in any of the studies; however one Dutch prospective cohort study (Leffelaar *et al*, 2010; n=3,730), reported that length of infants born to mothers with serum 25(OH)D concentration < 30 nmol/L compared to ≥ 50 nmol/L was significantly lower at 1 month and the infants were at higher risk of being SGA. Seven observational studies (4 cohort, 2 case-control & 1 cross-sectional study) assessed the relationship between maternal serum 25(OH)D concentration and risk of SGA. Three reported a significant association; out of these, 2 reported an inverse association while 1 (Bodnar *et al*, 2010), a nested case control study in the USA which included black and white women (from < 16 wks gestation) observed a U-shaped association among white mothers, with a significantly increased risk with serum 25(OH)D concentration < 37.5 nmol/L and > 75 nmol/L. There was no association between serum 25(OH)D concentration and SGA risk among black mothers. It has been suggested that an interaction between VDR genotype and serum 25(OH)D concentration, as also observed by Morley *et al* (2009; 2006) in an Australian cohort (see next paragraph), might explain differences between populations.
369. Burris *et al* (2012) examined the association between 2nd trimester cord plasma 25(OH)D concentration and SGA (n=1067 white & n=236 black mother infant pairs). Mean (SD) second trimester plasma 25(OH)D concentration was lower in black (46 ± 22 nmol/L) compared to white (62 ± 20 nmol/L) mothers. Mean (SD) cord plasma 25(OH)D concentration was also lower in black (31 ± 16 nmol/L) compared to white (51 ± 18 nmol/L) infants. Maternal plasma 25(OH)D concentration <25 vs >25 nmol/L was associated with higher odds of delivering an SGA infant (OR, 3.17; 95% CI, 1.16-8.63) and infants with cord plasma 25(OH)D concentration < 25 vs > 25 nmol/L had higher odds of being SGA (OR, 4.64; 95%CI, 1.61-13.36).

Maternal non-skeletal reproductive outcomes

370. Postulated adverse effects of low serum 25(OH)D concentration on maternal reproductive health include: gestational diabetes mellitus, pregnancy induced hypertension (PIH; also known as pre-eclampsia), increased risk of operative delivery, intra-hepatic cholestasis of pregnancy and periodontal disease in pregnancy (Brannon & Picciano, 2011; Finer *et al*, 2012; Dror, 2011).

Intervention studies

371. Two systematic reviews (De-Regil *et al*, 2012; Harvey *et al*, 2014) identified 1 RCT in India (Marya *et al*, 1987) that examined the effect of a combined vitamin D (30 µg/1200 IU/d) and calcium (375 mg/d) supplement on incidence of PIH and reported no significant effect (0.67; 95%CI, 0.33-1.35) but systolic and diastolic blood pressure was significantly lower in the treated group.

⁴¹ Both trials defined SGA as infants born below the 10th percentile for birth weight.

372. An RCT by Hollis *et al* (2011) (not included in either of the above reviews) found no statistically significant effect of daily vitamin D₃ supplementation (10 µg/400 IU; 50 µg/2000 IU; or 100 µg/4000 IU) on risk of instrumental delivery; however, there was no unsupplemented control group. A combined analysis of the studies by Hollis *et al* (2011) and Wagner *et al* (2012) also observed no difference in comorbidities of pregnancy by treatment group (Wagner *et al*, 2013); however, there were significantly fewer comorbidities when serum 25(OH)D concentration at delivery was > 80 nmol/L. The latter analysis was adjusted for study and ethnic group but not for other potential confounders.

Observational studies

373. A systematic review (Harvey *et al*, 2014) identified 11 observational studies (6 case-control, 4 cohort, 1 cross-sectional) on the effect maternal serum 25(OH)D concentration during pregnancy on PIH. Five of the studies (3 case-control, 1 cross-sectional, 1 cohort) reported significant associations between maternal serum 25(OH)D concentration and risk of PIH. Pooled results from 4 studies (Bodnar *et al*, 2007; Powe *et al*, 2010; Robinson *et al*, 2010; Azar *et al*, 2011) found no association between PIH risk and serum 25(OH)D concentration.

374. Harvey *et al* (2014) identified 8 observational studies (4 case-control, 1 cross-sectional, 3 prospective) on maternal serum 25(OH)D concentration and risk of gestational diabetes. The findings were inconsistent but the majority of studies found no association. No intervention studies were identified.

Later growth and development of the offspring

Maternal serum 25(OH)D concentrations in pregnancy, neonate/infant “stores” and breastfeeding

375. Maternal and neonatal serum 25(OH)D concentrations correlate at birth although cord blood concentration is lower than maternal concentration. This suggests that infants born to mothers with low serum 25(OH)D concentration have smaller ‘stores’ at birth than those born to mothers with a higher 25(OH)D concentration. The relatively few longitudinal data (none of UK origin) show that serum 25(OH)D concentration of breastfed infants falls with age and the correlation between maternal and cord concentration weakens (Brannon & Picciano, 2011). Studies are heterogeneous in outcome and the variance within studies is wide, making it difficult to attribute confidently a time course over which cord serum 25(OH)D concentration influences circulating serum 25(OH)D concentration during infancy. A figure of 8 weeks has been cited (Specker, 1994), assuming that the mother’s 25(OH)D concentration in pregnancy was sufficient, but can only be considered an approximation.

Intervention studies

376. A systematic review (Thiele *et al*, 2013) identified 3 RCTs (Hollis & Wagner, 2004; Wagner *et al*, 2006; Saadi *et al*, 2009) on the effects of maternal vitamin D supplement intake (10-160 µg/d) during lactation on serum 25(OH)D concentration of exclusively breast fed infants. The vitamin D content of breast milk increased significantly with vitamin D doses of 50-160 µg/d; no increase was observed with doses of 10 µg/d.

Observational studies

377. A longitudinal study in the US followed unsupplemented breastfed infants (n=35) for 18-months (Ziegler *et al*, 2006). Whilst plasma 25(OH)D concentration in some infants was > 25 nmol/L all year round, in others (n=22) it was < 12.5 nmol/L at some point (usually during the winter). The prevalence of plasma 25(OH)D concentration < 12.5 nmol/L fell with age but was still 12% at 15 months in infants not given supplements (n=25). Maternal plasma 25(OH)D concentration during pregnancy was not described and infants were not recruited until 28 days of age (earliest measurements at 112 days). The data were collected opportunistically during an iron supplementation study and no morbidity was ascribed nor any

radiographic examination performed. By comparison, a study in India (Jain *et al*, 2011) reported that 30% of breastfed infants (3 months of age) with plasma 25(OH)D concentration < 25 nmol/L showed radiological evidence of rickets despite absence of other clinical features or effect on growth.

378. Most observational studies which have examined serum 25(OH)D concentration of breast fed infants are not relevant to the UK (Merewood *et al*, 2012; Dawodu *et al*, 2012; Amukele *et al*, 2013 Green *et al*, 2012; Halicioglu *et al*, 2012); however, a cross-sectional study in New Zealand (Wall *et al*, 2013) showed significant seasonal variations in serum 25(OH)D concentration of healthy term exclusively breast fed infants (n=94; mean age, 10 weeks). Median serum 25(OH)D concentration was significantly lower (p=0.0001) in infants enrolled in winter (21 nmol/L; IQR⁴², 14-31 nmol/L) compared to those enrolled in summer (75 nmol/L; IQR, 55-100 nmol/L), autumn (49 nmol/L; IQR, 30-64 nmol/L) or spring (60 nmol/L; IQR, 40-79 nmol/L). Overall, 60% of infants whose serum 25(OH)D concentration was measured in winter had a concentration < 25 nmol/L compared with 4% in summer. Serum 25(OH)D concentrations were higher in spring than autumn, which was unusual. Since the study was cross-sectional, the effects over time of exclusive breast feeding on infant serum 25(OH)D concentration could not be assessed. Information was not available on sun exposure of the infant and maternal serum 25(OH)D concentration during the last trimester of pregnancy was not measured.

Later cognitive and psychological development

379. The Southampton Women's Survey (SWS), a prospective cohort study, did not find any associations between maternal serum 25(OH)D concentration in pregnancy and any tests of cognitive performance or psychological health to the age of 9 y (Gale *et al*, 2008). In another prospective cohort study in Western Australia, no associations were found between maternal serum 25(OH)D concentration and offspring psychological health at ages 2, 5, 8, 10, 14, and 17 years although there was a significant association between low serum 25(OH)D concentration in pregnancy and offspring language delay at the ages of 5 and 10 years (Whitehouse *et al*, 2012). Risk was approximately doubled in mothers with serum 25(OH)D concentration < 46 nmol/L in pregnancy compared to those with concentrations > 70 nmol/L.

380. There are several case reports in the literature of delayed motor milestones (principally delay in walking) among infants with clinical rickets (e.g., Agarwal *et al*, 2009) but no adequately controlled studies identifying effects of maternal or infant serum 25(OH)D concentration on infant development.

Maternal 25(OH)D concentration and later growth

381. An RCT (Brooke *et al*, 1981) has examined associations between ponderal and linear growth after birth (for 1 year) with maternal serum 25(OH)D concentration in pregnancy; vitamin D₂ supplementation (25 µg/1000 IU vitamin D₂ or placebo daily during last trimester) had no effect on head circumference.

382. In contrast, a prospective cohort study (SWS) observed a significantly greater head circumference at age 9 years in the offspring of mothers with serum 25(OH)D concentration > 75 vs < 30 nmol/L in the third trimester (Gale *et al*, 2008). It is possible this was a chance effect since no associations with any other childhood anthropometric measures were found.

Respiratory disease

383. Data from the UK SWS cohort to 9 years of age (Gale *et al*, 2008) found no association between maternal serum 25(OH)D concentration in pregnancy and increased cardio-respiratory risk (Gale *et al*, 2008). However, the offspring of mothers whose serum 25(OH)D concentration in pregnancy exceeded 75 nmol/L were at greater risk of eczema at 9 months and asthma at 9 years of age.

⁴² Interquartile range.

Summary - Pregnancy and lactation: non-skeletal outcomes in mother and baby

Non-skeletal health outcomes in the mother

384. One RCT in India reported no effect of a combined vitamin D and calcium supplement on incidence of PIH. One RCT reported no effect vitamin D supplementation on risk of instrumental delivery
385. Evidence from a systematic review on the association between maternal serum 25(OH)D concentration during pregnancy and PIH and gestational diabetes is inconsistent.

Non-skeletal health outcomes in the newborn

386. Evidence from 3 intervention trials indicate a reduction in neonatal hypocalcaemia incidence with maternal vitamin D supplementation in pregnancy. One observational study reported that a serum concentration < 25 nmol/L is associated with development of hypocalcaemia.
387. A systematic review of intervention trials indicates that vitamin D supplementation does not have beneficial effects on infant birth weight, birth length or risk of offspring being SGA or head circumference. Evidence from observational studies is inconsistent.
388. Data from longitudinal studies suggest that serum 25(OH)D concentration of breastfed infants falls with age. The time course over which fetal serum 25(OH)D concentration influences serum 25(OH)D concentration during infancy is not clear.
389. A systematic review of 3 RCTs reported a significant increase in the vitamin D content of breast milk with vitamin D doses of 50-160 µg/d but not 5 µg/d.
390. A cross-sectional study of exclusively breast-fed infants in New Zealand, found that 60% had a serum 25(OH)D concentration < 25 nmol/L in winter compared with 4% of infants in summer.
391. Findings from observational studies on associations between maternal serum 25(OH)D concentration and cognitive and psychological development of the offspring are inconsistent.

Cancers

392. Ecological studies have reported an increase in cancer risk with increasing latitude (IARC, 2009). Since the intensity of sun exposure decreases with increasing latitude, and on the basis that sun exposure is a proxy for vitamin D status, it has been suggested that vitamin D protects against cancer. This suggestion has been supported by cell culture studies (Halline *et al*, 1994; Cross *et al*, 1991) which found that 1,25(OH)₂D inhibited growth of malignant cell culture lines and reduced tumour development and growth in animal studies (Tangpricha *et al*, 2005; Mokady *et al*, 2000).

IOM report

393. The IOM reviewed the evidence on vitamin D and risk of all cancers, breast cancer, colorectal cancer and prostate cancer.
394. For all cancers, the IOM concluded that interpretation of the evidence was limited by the small number of studies, inconsistent associations and absence of large-scale RCTs. For colorectal cancer, it was noted that epidemiological studies, overall, suggested an inverse association with serum 25(OH)D concentration but that there was a paucity of randomised intervention studies and those that were available had not shown a significant benefit. For breast and prostate cancers, the IOM concluded that prospective studies showed inconsistent associations but RCTs were sparse. It concluded that the evidence on cancer was considered insufficient to support development of DRIs.

Evidence considered since IOM report

RCTs

395. Four RCTs have examined cancer risk in relation to supplemental vitamin D (Trivedi *et al*, 2003; Wactawski-Wende *et al*, 2006; Lappe *et al*, 2007; Avenell *et al*, 2012). A meta-analysis (Keum & Giovannucci, 2014) of these 4 trials (n=4333 cases; 45,151 participants) reported that vitamin D supplementation (10-27.5 µg/400-1100 IU/d) over 2-7 years had little effect on cancer risk (RR=1.00; 95% CI, 0.94-1.06; p=0.1).
396. A meta-analysis (Sperati *et al* 2013) of two of these RCTs (Lappe *et al*, 2007; Avenell *et al*, 2012) reported no effect of vitamin D supplementation (20-27.5 µg/d or 800-1100 IU/d) on risk of breast cancer (n=5372; RR=1.11; 95% CI, 0.74-1.68).

Cohort studies

397. Numerous prospective cohort studies have considered the relationship between serum 25(OH)D concentration and cancer risk. Most information is available for colorectal, breast and prostate cancers. These studies are, however, subject to confounding by behavioural and lifestyle factors that influence serum 25(OH)D concentrations. McCullough *et al* (2010) measured correlates of serum 25(OH)D concentration in a large control population of the 'Cohort consortium vitamin D pooling project of rarer cancers' covering a worldwide geographical area, including men and women from US, Chinese and Finnish cohorts. Statistically significant positive correlates of serum 25(OH)D concentration included male sex, vigorous physical activity and alcohol intake. Significant inverse correlates were BMI, diabetes, sedentary behaviour and smoking.
398. Colorectal cancer: A meta-analysis of 8 studies (Gandini *et al*, 2011) reported a significant inverse relationship between serum 25(OH)D concentration and colorectal cancer risk (RR = 0.85; 95% CI, 0.79-0.92 per 25 nmol/L increase in 25(OH)D). Out of 4 subsequent studies, 2 (Weinstein *et al*, 2011; Lee *et al*, 2011) reported that risk was non-significantly higher for participants in the highest category of serum 25(OH)D concentration and 2 (Neuhouser *et al*, 2012; Weinstein *et al*, 2014) reported a significantly lower risk for participants in the highest category of serum 25(OH)D concentration (see tables, Appendix 1 for serum 25(OH)D concentrations in these studies). Woolcott *et al* (2010) observed an inverse trend per

doubling of plasma 25(OH)D concentration (OR, 0.68; 95% CI, 0.51–0.92; p=0.01) although the results for analysis by quintiles of plasma 25(OH)D concentration showed that relative to the lowest quintile (< 42 nmol/L) colorectal cancer risk was significantly reduced only among participants with plasma 25(OH)D concentration in the third quintile (55 to < 66 nmol/L) (OR, 0.54; 95% CI, 0.32-0.93).

399. **Breast cancer:** A meta-analysis of 14 studies (Kim & Je, 2014) estimated that the risk of breast cancer incidence was lower for participants in the highest (> 77 nmol/L) vs lowest quintile (< 45 nmol/L) of serum/plasma 25(OH)D concentration (RR, 0.92; 95% CI, 0.83-1.02). A subsequent study (Kim *et al*, 2014) of five ethnic groups in the US (white, African-American, native Hawaiian, Japanese, Latino) reported an inverse association between breast cancer risk and 25 nmol/L increases in plasma 25(OH)D concentration ((OR, 0.66; 95% CI, 0.48-0.90) in whites but not in other ethnic groups.
400. **Prostate cancer:** A meta-analysis of 14 studies (Gilbert *et al*, 2011) estimated a reduction in prostate cancer per 25 nmol/L increase in serum 25(OH)D concentration (OR, 1.04; 95% CI, 0.99-1.10). Out of 5 subsequent studies, 2 (Meyer *et al*, 2013; Albanes *et al*, 2011) reported that higher serum 25(OH)D concentration was associated with significantly increased risk of prostate cancer. Meyer *et al* (2011) reported a rate ratio of 1.15 (95% CI, 1.04-1.27) per 30 nmol/L increase in serum 25(OH)D concentration while Albanes *et al* (2013) reported an OR of 1.56 (95% CI, 1.15-2.12) for men in the highest quintile of serum 25(OH)D concentration (> 45.6 nmol/L in winter; > 59.9 nmol/L in summer) compared with those in the lowest quintile (\leq 16.3 nmol/L in winter; \leq 25.9 nmol/L in summer). The other 3 studies (Brändstedt *et al*, 2012; Schenck *et al*, 2014; Kristal *et al*, 2014) found no significant associations. Shui *et al* (2012) observed a 57% reduction in risk of lethal prostate cancer in the highest versus lowest quartile of plasma 25(OH)D concentration⁴³ (OR, 0.43; 95% CI, 0.24-0.76), however there was no association with overall prostate cancer.
401. **Other cancers:** Less evidence is available for other cancers. No significant associations with serum 25(OH)D concentration were reported for cancers of the oesophagus and stomach combined (Abnet *et al*, 2010), larynx and oropharynx combined (Arem *et al*, 2011), lung (Kilkinen *et al*, 2008; Weinstein *et al*, 2011), endometrium (Zeleniuch-Jacquotte *et al*, 2010), ovary (Yin *et al*, 2011), kidney (Gallicchio *et al*, 2010; Mondul *et al*, 2014) or non-Hodgkin lymphoma (Purdue *et al*, 2010). Out of 2 studies on liver cancer (Fedirko *et al*, 2014; Wang *et al*, 2013), 1 (Fedirko *et al*, 2014) reported that serum 25(OH)D concentration <50 nmol/L vs \geq 75 nmol/L was associated with a 49% reduced risk of liver cancer (IRR⁴⁴ = 0.51; 95% CI, 0.26-0.99). Out of 2 studies on bladder cancer (Mondul *et al*, 2010; 2012), 1 (Mondul *et al*, 2010) reported that serum 25(OH)D concentration < 25 nmol/L vs \geq 50 nmol/L was associated with a significantly increased risk of bladder cancer (OR=1.73; 95% CI, 1.03-2.91) Three studies on melanoma reported a non-significantly higher risk in people with serum 25(OH)D concentration \geq 50 nmol/L compared to < 25 nmol/L (Major *et al*, 2012; Afzal *et al*, 2013) and \geq 75 compared to < 75 nmol/L (van der Pols *et al*, 2013). A meta-analysis (Caini *et al*, 2014) reported that higher serum 25(OH)D concentration was associated with a significant increase in risk in basal cell skin cancer (4 studies⁴⁵; RR = 1.82; 95% CI, 1.38-2.40) and non-melanoma skin cancer (2 studies⁴⁶; RR=1.64; 95% CI, 1.02-2.65). Stolzenberg-Solomon *et al* (2010) reported a significant increase in pancreatic cancer risk associated with higher (\geq 100 nmol/L) compared to lower (< 25 nmol/L) serum 25(OH)D concentration.

⁴³ Plasma 25(OH)D concentrations not specified.

⁴⁴ Incident rate ratio.

⁴⁵ Serum 25(OH)D concentrations in the top vs bottom quintile in the 4 studies were: >85 vs < 51; >78 vs <49; >37.4 vs <37; >75 vs <75.

⁴⁶ Serum 25(OH)D concentrations in the top vs bottom quintile in the 2 studies were (nmol/L): >50 vs <25; >75 vs <40.

Summary - Cancer

402. RCTs have not shown an effect of vitamin D supplements on overall cancer risk.
403. Observational studies indicate an inverse association between serum 25(OH)D concentration and colorectal cancer risk. This might be due to a protective effect, reverse causality, or residual confounding by other factors such as obesity, physical activity and smoking.
404. There is no strong evidence of associations between serum 25(OH)D concentration and risk of cancer at other sites. Although studies of skin cancer suggest risk may be increased in individuals with a relatively high serum 25(OH)D concentration this might be because a high serum 25(OH)D concentration is a marker of high sun exposure

Cardiovascular disease & hypertension

405. Cardiovascular disease (CVD) encompasses a range of diseases of the heart and circulation including coronary heart disease, angina, heart attack, congenital heart disease and stroke. There are several risk factors for CVD, including: smoking, high blood pressure, high blood cholesterol, physical inactivity, overweight, diabetes, family history, sex, age⁴⁷.
406. CVD is predominantly caused by atherosclerotic deposits in large and medium size arteries. These deposits are characterised by lipid deposition and inflammation. In addition, calcification is a significant component of advanced atherosclerotic lesions. It is important, therefore, to also consider the impact of calcium intake in relation to atherosclerotic CVD. The decline in CVD mortality over the last 20 years has been mainly due to better medical treatment for CVD and its associated risk factors.
407. Several lines of evidence have been suggested in support of a biologically plausible relationship between serum 25(OH)D concentration and cardiovascular events. Animal studies have shown that VDR knockout mice develop heart failure despite normalised calcium concentrations (Bouillon *et al*, 2008). Animal studies have also suggested a link between ingested vitamin D and atherosclerosis (Kunomita *et al*, 1981; Toda *et al*, 1983 & 1985; Peng *et al*, 1978). It has been proposed (Fraser, 2011; *personal communication*) that this is because vitamin D is metabolised to 25(OH)D in the liver at the same time as the dietary triglycerides are being repackaged as very-low density lipoprotein (VLDL) particles which are secreted back into the circulation with some 25(OH)D incorporated into the VLDL; since endothelial cells have specific receptors for lipoproteins, the uptake of LDL could deliver 25(OH)D to these cells.
408. Ecological studies have reported increased CVD mortality and hypertension at more Northern latitudes and during the winter (Grimes *et al*, 1996; Rostand, 1997; Zitterman *et al*, 2005). There is evidence suggesting that inflammatory processes are involved in the development of CVD (van Lente, 2000) and the potential role of vitamin D in modulating inflammation (see section x) has been proposed as a possible mechanism providing linkage to CVD. PTH concentration is inversely correlated with serum 25(OH)D concentration and epidemiological studies have demonstrated an association between elevated and high-normal PTH concentration and increased risk of cardiovascular events and mortality (Pilz *et al*, 2010; van Ballegooijen *et al*, 2013); PTH suppression by vitamin D supplementation might therefore reduce CVD risk.
409. Since vitamin D has the potential to increase calcium absorption in the presence of high calcium intakes, it is also biologically plausible that vitamin D might increase vascular calcification and as a consequence, increase CVD risk.

IOM report

410. The IOM report was unable to identify any RCTs that examined CVD as a pre-specified primary outcome. Several trials analysed CVD as a secondary outcome but did not find a reduction in CVD risk with vitamin D supplementation. Observational studies supported a relationship between serum 25(OH)D concentration and presence of CVD but not risk for developing CVD. The IOM Committee concluded that it could not draw an inference about the efficacy of this indicator to support DRI development.

⁴⁷ British Heart Foundation (<https://www.bhf.org.uk/heart-health/conditions/cardiovascular-disease>).

Evidence considered since the IOM report

Systematic reviews and meta-analyses

CVD

Intervention studies

411. RCT data on vitamin D supplementation and CVD are derived mainly from studies designed to evaluate effects of vitamin D supplementation on musculoskeletal outcomes and need, therefore, to be interpreted with caution.
412. A systematic review of 8 randomised trials with CVD as a secondary outcome (Wang *et al*, 2010) reported no significant associations for CVD with vitamin D supplementation (supplemental daily doses of approximately 25 µg/1000 IU; pooled RR, 0.90; 95% CI, 0.77–1.05), calcium supplementation or a combination of vitamin D plus calcium supplementation.
413. In contrast, a meta-analysis of 3 placebo controlled trials (Bolland *et al*, 2011) reported that calcium and vitamin D increased the risk of myocardial infarction (RR, 1.21; 95% CI, 1.01-1.44; p=0.04), stroke (RR, 1.20; 95% CI, 1.00-1.43; p=0.05) and the composite of myocardial infarction or stroke (RR, 1.16; 95% CI, 1.02-1.32; p=0.02).
414. Another systematic review (Ford *et al*, 2014) of 21 RCTs (n=13,033; mean/median age ≥ 60 years; ≥ 1 year follow-up) reported that estimated HRs (95% CIs) for vitamin D compared with placebo or control for cardiac failure, myocardial infarction and stroke were not significant: 0.82 (0.58-1.15), 0.96 (0.83-1.10) and 1.07 (0.91-1.29) respectively.

Cohort studies

415. A systematic review and meta-analysis (Grandi *et al*, 2010) included 9 prospective studies on vitamin D and CVD (4 on non-fatal CVD events & 5 on CVD mortality). Two out of the 4 studies on CVD risk reported a significantly increased risk in participants with serum 25(OH)D concentration < 37 nmol/L; meta-analysis of these studies supported an overall association of serum 25(OH)D concentration in the lowest (< 37 nmol/L) compared to the highest (> 75 nmol/L) categories with cardiovascular events (pooled HR=1.54; 95% CI, 1.22-1.95). Meta-analysis of the 5 studies with CVD mortality outcomes indicated a significantly increased risk (hazard ratio=1.83; 95% CI, 1.19–2.80) in individuals with serum 25(OH)D concentration below cut-offs ranging from approximately 20 to 45 nmol/L; significant heterogeneity was detected among studies (Q=21.01; p=0.0003).
416. Pilz *et al* (2011) summarised population-based prospective cohort studies examining the association between serum 25(OH)D concentration and cardiovascular events and mortality; the studies are not consistent but serum 25(OH)D concentrations were below cut-offs ranging from < 26 to < 38 nmol/L in those studies that reported a significant association with increased CVD risk.
417. Another meta-analysis of 19 prospective cohort studies (Wang *et al*, 2012) reported an inverse association between baseline serum 25(OH)D concentration with considerable heterogeneity between studies. The pooled relative risk of lowest to highest 25(OH)D concentration was 1.52 (95% CI: 1.30-1.77). CVD risk increased with decreasing serum 25(OH)D concentrations below approximately 60 nmol/L (RR, 1.03; 95% CI, 1.00-1.06 per 25 nmol/L decrement in 25(OH)D concentration).
418. Out of 6 subsequent prospective studies, 2 reported no association between serum 25(OH)D concentration and CVD incidence (Messenger *et al*, 2012; Welsh *et al*, 2012) while 4 found an association (Karakas *et al*, 2013; Robinson-Cohen *et al*, 2013; Kuhn *et al*, 2013; Perna *et al*, 2013). Karakas *et al* (2013) reported a decreased risk of coronary heart disease (CHD) associated with serum 25(OH)D concentrations

> 47.7 nmol/L, which was significant in women (HR, 0.42; 95% CI, 0.19-0.93; $p=0.028$). Robinson-Cohen *et al* (2013) reported that lower 25(OH)D concentrations were associated with increasing CHD risk in white (HR, 1.26; 95% CI, 1.06-1.49 per 25 nmol/L decrement) and Chinese (HR, 1.67; 95% CI, 1.06-1.49 per 25 nmol/L decrement) participants but not in black and Hispanic participants. Kuhn *et al* (2013) reported that serum 25(OH)D concentration < 25 nmol/L compared to ≥ 50 nmol/L was significantly associated with increased risk of MI, stroke and CVD as a composite endpoint (HR, 1.53; 95% CI, 1.12–2.09). Perna *et al* (2013) reported an increased risk of CVD with serum 25(OH)D concentration below 75 nmol/L.

Hypertension

Intervention studies

419. Studies assessing the effect of vitamin D supplementation on blood pressure are generally small and results have been inconsistent. Meta-analyses of vitamin D supplementation studies have included different studies and reached different conclusions. Witham *et al* (2009) included 11 RCTs which were small and of variable methodological quality. Meta-analysis of 8 studies which included participants with mean baseline blood pressure > 140/90 mm Hg showed a small, statistically significant reduction in diastolic blood pressure (-3.1 mm Hg, 95% CI, -5.5 to -0.6). Blood pressure was not reduced in studies with participants who were normotensive at baseline.
420. Wu *et al* (2010) included 4 double blind RCTs of normotensive and hypertensive individuals ($n=429$). Vitamin D supplementation significantly reduced systolic blood pressure by 2.44 mm Hg (95% CI, -4.86, -0.02) but not diastolic blood pressure. Change of blood pressure did not vary markedly across the dose of vitamin D supplementation, study length, or intervention.
421. Pittas *et al* (2010) found no effect of vitamin D supplementation on blood pressure in a meta-analysis of 10 trials. Another meta-analysis (Kunutsor *et al*, 2014) of 16 RCTs showed a non-significant reduction in systolic (-0.94; 95% CI, -2.98-1.10 mmHg) and diastolic (-0.52; 95% CI, -1.18, 0.14 mm Hg) blood pressure, with evidence of heterogeneity ($I^2 = 67.9\%$, $p < 0.001$) and publication bias ($p = 0.02$) among trials of systolic blood pressure. There was a significant reduction in diastolic blood pressure (-1.31, 95% CI -2.28, -0.34 mm Hg, $P = 0.01$) in participants with pre-existing cardiometabolic disease. The lack of consistent findings in these meta-analyses, suggest the relative weakness of the data.

Cohort studies

422. Observational studies suggest an inverse association between serum/plasma 25(OH)D concentration and hypertension. A meta-analysis (Burgaz *et al*, 2011) which included 18 studies (4 prospective and 14 cross-sectional) reported a pooled odds ratio for hypertension of 0.73 (95% CI, 0.63-0.84) for the highest versus lowest category of serum/plasma 25-hydroxyvitamin D concentration. In a dose-response meta-analysis, the odds ratio for a 40 nmol/L (approximately 2 SDs) increment in serum/plasma 25(OH)D concentration was 0.84 (95% CI, 0.78-0.90).
423. A subsequent prospective cohort study of men in the US ($n=1211$) followed up for 15 years, reported that compared with men in the lowest quartile of plasma 25(OH)D concentration, those in the third quartile had a significantly lower risk of incident hypertension (HR, 0.69; 95% CI, 0.50-0.96). Men in the highest quartile, however, did not have further reduced risk of hypertension (HR, 0.82; 95% CI, 0.60-1.13).

Summary - CVD

424. Intervention studies have generally considered CVD risk as a secondary outcome; these studies, therefore, need to be interpreted with caution.
425. Out of 3 systematic reviews assessing the effect of vitamin D supplementation on CVD outcomes, 2 reported no significant effect and 1 reported an increased CVD risk with vitamin D plus calcium.
426. Prospective cohort studies, overall, report an inverse association between serum 25(OH)D concentration and CVD risk. Increased risk in these studies was reported at serum 25(OH)D concentrations ranging between < 25 nmol/L and 60 nmol/L.
427. Meta-analyses of intervention studies on vitamin D supplementation and hypertension are inconsistent.
428. Observational studies (cohort and cross-sectional) report an inverse association between serum 25(OH)D concentration and hypertension.

All-cause mortality

429. An effect of vitamin D on mortality risk has been suggested in numerous observational studies reporting associations between low serum 25(OH)D concentration and increased risk of several chronic diseases (including CVD and cancer) and because vitamin D has been implicated in modulating inflammatory and immune processes.

IOM report

430. The IOM only considered all-cause mortality in the context of adverse effects of excess vitamin D. The report concluded that the data were suggestive of a U-shaped or reverse J-shaped relationship between serum 25(OH)D concentration and all-cause mortality with an increase in risk suggested at concentrations < 30 and > 75 nmol/L.

Evidence since IOM report

Intervention studies

431. A Cochrane systematic review and meta-analysis (Bjelakovic *et al*, 2014) included 56 randomised trials of any form of vitamin D (n=95,286; mean treatment duration, 4.4 y); 34 trials used vitamin D in combination with calcium in the intervention group. Participants in most of the trials were women aged over 70 years (a population at greater risk of mortality) and many of the trials were small, with less than 10 deaths. Overall, treatment with any type of vitamin D decreased mortality (RR= 0.97; 95% CI, 0.94-0.99; p=0.02). In analyses of vitamin D given without calcium, vitamin D₃ vs placebo or no intervention (13 trials; n=12,609) had no statistically significant effect on mortality (RR=0.92; 95% CI, 0.85-1.00; p=0.06); vitamin D₂ administered alone (8 trials; n=17,079) also had no statistically significant effect on mortality (RR=1.03; 95% CI, 0.96-1.12).

Cohort studies

432. A meta-analysis of observational prospective studies (Schöttker *et al*, 2014) which combined individual participant data from 8 cohorts in the USA and Europe (n=26,018; age, 50-79 y) estimated a pooled hazard ratio of 0.64 (95% CI, 0.55-0.74) for the highest versus the lowest fifth of serum 25(OH)D concentration. Cut-offs for serum 25(OH)D quintile concentrations varied by cohort ranging from < 16 to < 42 nmol/L in the lowest quintile and from ≥ 44 to ≥ 86 nmol/L in the highest.

433. Another meta-analysis of observational cohort studies (Chowdhury *et al*, 2014) summarised results from 27 cohorts (n=780,990). The pooled hazard ratio was 0.74 (95% CI, 0.67-0.82) for the highest versus the lowest third of serum 25(OH)D concentrations (median concentration 52 nmol/L; interquartile range, 44-61 nmol/L).

434. A subsequent prospective cohort study of community dwelling adults in Spain (n=328, 85+ y) followed for 3 years, found no association between serum 25(OH)D concentration and overall mortality (Formiga *et al* 2014). Another study in Australia (n=4203 men, 1144 deaths, age 70-88 y) reported an association between plasma 25(OH)D concentration < 52.9 nmol/L and all-cause mortality (HR, 1.20; 95% CI, 1.02-1.42) which was independent of baseline frailty (Wong *et al*, 2013). In an analysis of NHANES III data (n=15099, 3784 deaths, age, ≥ 20 years) with 15 years follow-up (Sempos *et al*, 2013), serum 25(OH)D concentration < 75-99 nmol/L (reference category) was associated with significantly increased mortality risk (RR, 1.5; 95% CI, 1.2-1.8 for serum 25(OH)D < 20 nmol/L). A significantly increased risk was also found

at serum 25(OH)D concentrations ≥ 120 nmol/L (J-shaped association) in a minimally adjusted model⁴⁸ (RR, 1.5; 1.02-2.3) but the association was attenuated after further adjustment⁴⁹ (RR, 1.4; 95% CI, 0.96-2.2).

Summary - mortality

435. Evidence from a systematic review of 56 randomised trials shows that vitamin D supplementation in combination with calcium reduces mortality risk but, overall, vitamin D supplementation alone does not affect total mortality.
436. Evidence from 2 meta-analyses of observational studies indicate an inverse association between serum 25(OH)D concentration and mortality. This might be due to a protective effect of vitamin D on mortality risk, reverse causality, or residual confounding by other factors such as obesity, physical activity and smoking.

⁴⁸ Age, sex, race-ethnicity, season

⁴⁹ Age, sex, race-ethnicity, season, self-reported diabetes, congestive heart failure, stroke, heart attack, cancer, glomerular filtration rate, BMI, physical activity, smoking, education, medication use.

Autoimmune disease

437. Autoimmune disease is characterised by the production of antibodies against the body's own tissues. The aetiology and pathogenesis of most autoimmune disorders remains unknown and a number of factors have been implicated in their development. Since ecological studies have reported a variation in prevalence of certain autoimmune diseases with latitude, it has been proposed that sunlight exposure, and therefore vitamin D, may play a role in their development.
438. VDRs are expressed in cells of the immune system (e.g., lymphocytes, macrophages, natural killer cells) suggesting that vitamin D may have immunomodulatory effects. Ecological data suggest greater prevalence of several autoimmune diseases in Northern latitudes where sun exposure is lower (Ponsonby *et al*, 2002; Staples *et al*, 2003). A number of epidemiological studies have reported an association between low serum 25(OH)D concentration and several autoimmune disorders including asthma, atopic dermatitis, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus (Kriegel *et al*, 2011; Antico *et al*, 2012).

IOM report

439. The IOM concluded that, overall, the evidence was not consistently supportive of a causal role for vitamin D in reducing risk of autoimmune disease.

Evidence considered since IOM report

440. A systematic review of 219 studies (cross-sectional, intervention and prospective) examined whether serum 25(OH)D concentration was related to the risk of developing autoimmune disease and whether vitamin D supplementation could modify its progress (Antico *et al*, 2012). Most of the studies were of patients with pre-existing autoimmune disease; only 2 prospective cohort studies examined correlations between serum 25(OH)D concentration and risk of developing autoimmune disease. Out of these, 1 (Nielen *et al*, 2006) found no correlation between serum 25(OH)D concentration and risk of developing rheumatoid arthritis while the other (Munger *et al*, 2006) found an inverse association between risk of developing multiple sclerosis and serum 25(OH)D concentration in white participants only (OR per 50 nmol/L increase, 0.59; 95% CI, 0.36-0.97). The authors conclude that although genetic and epidemiological studies suggest a possible role of vitamin D in the prevention of auto-immune diseases there is little evidence of benefit from vitamin D supplementation.

Asthma and atopic disorders

Intervention studies

441. A randomised trial examined the effect of prenatal vitamin D supplementation on childhood wheezing (Goldring *et al*, 2013). Pregnant women (n=180; at 27 weeks gestation) were randomised to receive either vitamin D₂ (20 µg/800 IU daily), a single dose of vitamin D₃ (5000 µg/200,000 IU), or no vitamin D. There was no significant difference between groups in risk of wheeze at 3 years of age (HR, 0.86; 95% CI, 0.49-1.50; p=0.69).

Cohort studies

442. A systematic review on the effects of vitamin D supplementation in pregnancy on multiple maternal and childhood health outcomes, which identified 8 observational studies assessing relationships between maternal vitamin D intake during pregnancy (n=4), maternal serum 25(OH)D concentration in pregnancy (n=2) or cord blood 25(OH)D concentration (n=2) and asthma, reported conflicting results (Harvey *et al*, 2014). The authors highlighted that substantial heterogeneity in study design, outcome and exposure definition and the conflicting results made it difficult to conclude any definitive relationship between maternal serum 25(OH)D concentration and development of asthma in the offspring.

443. Tolppanen *et al* (2013) examined prospective associations between serum 25(OH)D₂ and 25(OH)D₃ concentration measured in children aged 9.8 years incidence of wheezing (n=3323, 141 cases), asthma (n=3323, 464 cases) and flexural dermatitis (n=3748, 300 cases). Serum 25(OH)D₂ concentration was inversely associated with wheezing (OR per doubling of exposure, 0.83, 95% CI, 0.68–1.00) and flexural dermatitis (OR, 0.83; 95% CI, 0.72–0.94) and serum 25(OH)D₃ concentration was positively associated with wheezing (OR, 1.14; 95% CI, 1.03–1.28) and flexural dermatitis (OR, 1.09; 95% CI, 1.00–1.18).
444. A nested case control study (Mai *et al*, 2012) found no association between baseline serum 25(OH)D concentration < 50 nmol/L and asthma in men (OR 1.47; 95% CI, 0.93-2.32) or women (OR, 0.94; 95% CI, 0.67-1.32).
445. A prospective study in Australia (Hollams *et al*, 2011) reported that serum 25(OH)D concentration in children at age 6 y (n=989) was inversely associated with the risk of developing atopy (OR, 0.14; 95% CI, 0.04-0.47) and asthma (OR, 0.11; 95% CI, 0.02-0.84) at age 14 y (n=689).
446. Chawes *et al* (2014) investigated the relationship between cord serum 25(OH)D concentration and development of asthma and allergy-related conditions in early childhood (n=257). Children were followed until the age of 7 y and were monitored for troublesome lung symptoms (TROLS), asthma, respiratory infections, allergic rhinitis and eczema. After adjustment for season of birth, cord serum 25(OH)D concentration < 50 nmol/L was associated with a significant increase in risk of recurrent TROLS (HR, 2.65; 95% CI, 1.02-6.86). No association was found with respiratory infections or asthma, lung function, rhinitis or eczema.

Atopic Disorders

Intervention studies

447. No intervention studies could be identified.

Cohort studies

448. The systematic review by Harvey *et al* (2014) (see paragraph 442) also examined the relationship between serum 25(OH)D concentration in pregnancy and atopy risk in offspring. Out of 2 studies which conducted skin prick tests as a measure of atopic sensitisation, one showed no effect of maternal serum 25(OH)D concentration on atopic sensitisation to potential allergens at 5 years of age (Devereux *et al*, 2007) while the other showed a greater risk of a positive response to a number of allergens when cord plasma 25(OH)D concentration was ≥ 100 nmol/L compared to concentrations between 50-74.9 nmol/L (Rothers *et al*, 2011). This study also demonstrated a non-linear relationship between cord plasma 25(OH)D concentration and total and allergen-specific IgE for 6 allergens, with the highest IgE concentrations in children with cord plasma 25(OH)D concentration < 50 nmol/L and ≥ 100 nmol/L.
449. Jones *et al* (2012) prospectively examined the association between vitamin D exposure *in utero* and allergic outcomes in the first year of life in mother-infant pairs (n=231). Cord blood 25(OH)D concentration was significantly lower in infants who developed eczema by 12 months of age (p=0.18). Risk of eczema was significantly reduced with increasing cord blood 25(OH)D concentration: a 10 nmol/L increase in cord blood 25(OH)D concentration significantly reduced risk by risk by 13.3% (OR, 0.87; 95% CI, 0.77-0.98; p=0.02).
450. Weisse *et al* (2013) measured serum 25(OH)D concentration in mother-child pairs (n=378) during pregnancy and at birth. In a multivariate regression model, maternal serum 25(OH)D concentration was positively associated with children's risk of food allergy within the second year of life (OR, 3.66; 95% CI, 1.36-9.87) or within the 2 year lifetime period (OR, 1.91; 95% CI, 1.09-3.37). Higher maternal serum 25(OH)D concentration (was also associated with a greater risk of sensitisation against food allergens (OR,

1.59; 95% CI, 1.04-2.45⁵⁰). Cord serum 25(OH)D concentration was also positively associated with the children's risk of food allergy within the second year of life (OR, 4.65; 95% CI, 1.5-14.48). No association was found between cord serum 25(OH)D concentration and food allergy within the first year of life or for atopic eczema, total IgE or specific IgE at all time points.

Type I Diabetes Mellitus

Intervention studies

451. No intervention studies could be identified.

Cohort studies

452. Simpson *et al* (2011) investigated the association between serum 25(OH)D concentration and development of islet autoimmunity (IA) and type I diabetes in children (n=2644; age, 9 m-10 y) at increased risk of type 1 diabetes. Over 8 years of follow-up, 198 children developed IA but there was no association between serum 25(OH)D concentration and risk of developing IA or type I diabetes.
453. A prospective nested case control study among US active-duty military personnel identified type I diabetes cases (n=310) with at least 2 serum 25(OH)D samples collected before disease onset and controls (n=613) (Munger *et al*, 2012). Non-Hispanic whites with serum 25(OH)D concentration \geq 100 nmol/L had a 44% lower risk of developing type I diabetes compared to those with concentrations $<$ 75 nmol/L (RR, 0.56; 95% CI, 0.35-0.90; p=0.03). No significant association was found in non-Hispanic blacks or Hispanics.
454. A prospective nested case-control study in Norway examined whether lower maternal serum 25(OH)D concentration during pregnancy was associated with an increased risk of childhood-onset type 1 diabetes (Sørensen *et al*, 2012). Mean serum 25(OH)D concentration in pregnant women (cases; n=109) who delivered a child that subsequently developed type I diabetes before the age of 15 years was compared with controls (n=219). Mean serum 25(OH)D concentration was significantly lower in cases than in controls (65.8 vs 73.1 nmol/L; p=0.021). Offspring of cases in the lowest quartile of 25(OH)D concentration (\leq 54 nmol/L) were at a higher risk of developing type I diabetes compared to those in the upper quartile ($>$ 89 nmol/L) (OR, 2.38; 95% CI, 1.12-5.07; p=0.03).

Inflammatory Bowel Disease (IBD)

455. IBD is a group of chronic inflammatory conditions that affect the gastrointestinal tract and mainly includes ulcerative colitis and **Crohn's disease**. The IOM report did not identify any systematic reviews or RCTs for this indicator. It noted that 2 cross-sectional analyses had evaluated serum 25(OH)D concentrations of patients with IBD (Pappa *et al*, 2006; Jahnsen *et al*, 2002) but the study design diminished the reliability of the findings. These studies were not able to assess whether serum 25(OH)D concentration influences the development of this condition.
456. No studies published after the IOM report, on vitamin D and risk of IBD could be identified. An RCT in Denmark assessed the effect of oral vitamin D₃ treatment on clinical relapse in patients (n=94) with Crohn's disease in remission. Patients were randomised to receive either vitamin D₃ (30 µg/1200 IU per day) or placebo for 12 months. The serum 25(OH)D concentration of patients in the vitamin D treatment group increased from mean (SD) 69±31 nmol/L to 96±27 nmol after 3 months (p< 0.001). The relapse rate was lower in the vitamin D₃ treated group compared with the placebo group (13% compared to 29%; p=0.06).

⁵⁰ 4th quartile (25th-75th percentile: 80-152 nmol/L) vs 1st quartile (25th - 75th percentile: 15-36 nmol/L).

Multiple Sclerosis

IOM Report

457. The IOM report noted that low solar exposure, latitude and polymorphisms in the *Vdr* gene have been implicated in susceptibility to multiple sclerosis (MS) but observational studies for an association between MS and vitamin D were inconsistent and no RCTs could be identified. It concluded that the lack of causal evidence diminished the likelihood for a relationship between vitamin D and MS.

Evidence considered since IOM report

Cohort studies

458. A prospective study in Sweden (Salzer *et al*, 2012) examined the association between serum 25(OH)D concentration and risk of MS in blood samples collected prospectively and during gestation. In the identified cases (n=182) median time from sampling to MS onset was 9 years. Serum 25(OH)D concentration ≥ 75 nmol/L was associated with a decreased risk of MS (OR, 0.39; 95% CI, 0.16-0.98). No association was found between gestational serum 25(OH)D concentration and MS risk in offspring.

Genetic studies

459. Huang and Xie (2012) and Tizaoui *et al* (2014) conducted meta-analyses of case-control studies investigating the association between *Vdr* polymorphisms and risk of MS. Huang and Xie (2012) reported that the Apal, Bsml, FokI and TaqI polymorphisms were not associated with MS risk. Tizaoui *et al* (2014) reported a significant association between the Apal polymorphism and MS pathogenesis but this was only observed in two of the genetic models (homozygous and codominant); the FokI polymorphism was significantly associated with MS but only after exclusion of one of the studies following sensitivity analysis. It was also noted that the FokI polymorphism influences *Vdr* protein structure but that Apal does not.
460. Ramagopalan *et al* (2011) performed whole exome sequencing of individuals (n=43) with MS from families with 4 or more MS-affected individuals and identified a rare variant of CYP27B1. From subsequent genotyping in other populations, they concluded that the rare variant was associated with risk of MS. This variant is rare in the population and by itself cannot account for most cases of MS but the authors note that CYP27B1 encodes the vitamin D-activating 1-alpha hydroxylase enzyme and that the identified variant has functional effects on 1,25(OH)₂D and risk of rickets; because of this, they interpret their findings as supporting a causative role for vitamin D in MS.

Rheumatoid Arthritis

IOM Report

461. The IOM concluded that there were no large prospective studies and no clinical trials to support a relationship between vitamin D and incidence of rheumatoid arthritis

Studies considered since IOM report

Intervention studies

462. Postmenopausal women (n=36,282) in the Women's Health Initiative Study of calcium and vitamin D were randomised to receive vitamin D₃ (10 µg/400 IU) plus calcium (1,000 mg) daily or placebo (Racovan *et al*, 2011). Over an average of 5 years, 163 new cases of rheumatoid arthritis were identified (by self-report and validated rheumatic medication use) but there was no difference between the two groups (HR, 1.04, 95% CI, 0.76-1.41).

Systemic lupus erythematosus (SLE)

IOM Report

463. The IOM reported that no RCTs or meta-analyses could be identified for this indicator. Observational studies which suggested an association between vitamin D and SLE showed variability in serum 25(OH)D concentrations associated with SLE. The IOM concluded that the evidence was not sufficient to permit conclusions being drawn about an association between SLE and serum 25(OH)D concentration.
464. No further studies on serum 25(OH)D concentration and risk of SLE could be identified.

Summary – Autoimmune disease

465. A large systematic review (219 intervention, prospective and cross-sectional studies) reported that vitamin D supplementation has little effect on risk of developing auto-immune disease.
466. There is a paucity of RCTs on the effect of vitamin D supplementation on development of specific autoimmune diseases. One RCT reported no effect of vitamin D supplementation during pregnancy on childhood wheezing.
467. Evidence from cohort studies on maternal serum 25(OH)D concentration and development of asthma in the offspring is inconsistent. A systematic review of observational studies reported that conflicting results make it difficult to establish any clear relationship. Findings from 4 subsequent cohort studies are inconsistent.
468. Findings from cohort studies of atopic disorders are inconsistent.
469. Evidence to link vitamin D and MS is largely observational and inconsistent. Genetic studies suggest associations between the Apal and FokI VDR polymorphisms and MS risk but the role of CYP27B1 in the development of MS is unclear.
470. Data are lacking on the relationship between vitamin D and type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis and SLE.

Infectious disease

471. A possible role of vitamin D in modulating the immune response has been suggested by the presence of VDRs and 1 α -hydroxylase (CYP27B1) in various cells of the immune system including B and T lymphocytes, macrophages and dendritic cells. Cell studies have shown that vitamin D enhanced bactericidal activity of human macrophages against *Mycobacterium tuberculosis* (Crowle *et al*, 1987). Liu *et al* (2006) reported that toll-like receptor (TLR⁵¹) activation of human macrophages upregulated expression of the VDR and 1 α -hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular *M tuberculosis*. It has been proposed that in vitamin D deficiency, the infected macrophage is unable to produce sufficient 1,25(OH)₂D to upregulate production of cathelicidin.
472. Support for an immunomodulatory role has also been suggested by ecological studies showing associations between seasonal variations in serum 25(OH)D concentrations and incidence of various infectious diseases including respiratory infection (Grant, 2008) and influenza (Cannell *et al*, 2006).
473. Possible mechanisms for the how vitamin D may play a role in host resistance to pathogens is considered further in a review by Lang *et al* (2013). The review concludes that current epidemiological data suggest that vitamin D deficiency increases susceptibility to various pathogens, however the underlying mechanisms still require clarification and further investigation, including the role of inherited polymorphisms in DBP, CYP27B1 and VDR genes.

IOM report

474. The IOM considered the effect of vitamin D on tuberculosis, influenza and upper respiratory infections. The report noted that results from RCTs and observational studies were inconsistent and that prospective studies were limited by potential confounding. It concluded that overall the evidence was not consistently supportive of a causal role for vitamin D in reducing the risk of developing infectious disease.

Evidence considered since IOM report

475. Most of the evidence on vitamin D and infection relates to use of vitamin D as a therapeutic agent in patients with pre-existing disease and considers whether vitamin D supplementation reduces severity or progression of the disease. Evidence is lacking on whether vitamin D supplementation can influence the risk of developing infectious disease.
476. Another difficulty that complicates interpretation of the data on vitamin D and infection is findings from a study by Reid *et al* (2011) which reported that plasma 25(OH)D concentration is decreased during acute inflammation (see paragraph 134) which arises in response to infection.

Tuberculosis

477. Tuberculosis (TB) is an infection caused by the bacterium *Mycobacterium tuberculosis* which typically affects the lungs. It is transmitted by inhalation of airborne particles. A person exposed to TB will not necessarily develop the disease as the immune system is usually able to destroy the bacteria. Sometimes the immune system can stop the infection from spreading but is unable to remove it from the body where it remains in an inactive or latent state. If the host immune system is unable to destroy or contain the infection, it can spread to the lungs or other parts of the body and symptoms develop within a few weeks or months; this is known as active TB. Latent TB may develop into active disease at a later date, particularly if the immune system becomes weakened.

⁵¹ Recognition of microbial components by TLRs initiates signal transduction pathways, which triggers expression of genes. These gene products control innate immune responses and further instruct development of antigen-specific acquired immunity.

RCTs

478. Most RCTs have tested whether vitamin D therapy improves TB outcomes and might be used as an adjunctive treatment. No RCTs of vitamin D supplementation for the prevention of active TB in those with a latent infection could be identified.

Observational studies

479. Several studies have shown a seasonal pattern associated with TB incidence, peaking in spring and being lowest in autumn. For example, a study assessing patterns of TB seasonality in New York City (Parrinello *et al*, 2012) reported that incidence was highest in March-May (27%) and lowest in September-November (22%). Although lower serum 25(OH)D concentration during winter was suggested as a possible cause of this seasonal pattern, another suggested cause was increased crowding in winter.
480. A number of observational studies in different populations have reported an association between low serum 25(OH)D concentration and increased risk of TB. A systematic review and meta-analysis of 7 observational studies (3 prospective; 4 case-control; n=531) (Nnoaham & Clarke, 2008) reported a 70% probability that a healthy individual without TB would have a higher serum 25(OH)D concentration than an individual with TB. The authors concluded that low serum 25(OH)D concentration increased the risk of active TB, however serum 25(OH)D concentrations in individuals with TB ranged from a median of 16 (2.25-74.25) to 65.8 (43.8-130.5) nmol/L.
481. A prospective cohort study in Spain examined the relationship between serum 25(OH)D concentration and incidence of TB among contacts of TB patients (n=572) who were followed up for 3 years (Arnedo-Pena *et al*, 2015). Mean serum 25(OH)D concentration was 34 nmol/L for cases and 64 nmol/L for non-cases. An inverse association was found between serum 25(OH)D concentration and TB incidence (adjusted HR, 0.88; 95% CI, 0.80-0.97).
482. Fewer studies have investigated latent TB infection (LTBI). Arnedo-Pena *et al* (2011) examined the association between serum 25(OH)D concentration and LTBI prevalence and tuberculin skin test (TST) conversion⁵² in contacts of TB patients (n=202). In a cross-sectional analysis, no association was found between participants identified with LTBI (n=50) and serum 25(OH)D concentration. After 2 months, 11 out of 93 negative LTBI participants, presented with TST conversions. Serum 25(OH)D concentration > 75 nmol/L was associated with a protective effect against TST conversion (OR of < 50 vs > 75 nmol/L=0.10; 95% CI, 0.00-0.76); however, the size of the study in relation to TST conversion is small and TST has low specificity with the possibility of false results (Mancuso *et al*, 2008).

Genetic studies

483. A systematic review and meta-analysis of 8 studies which examined associations between polymorphisms in the VDR gene (FokI, TaqI) and susceptibility to TB (Lewis *et al*, 2005) reported that results were inconclusive. The authors noted that the studies were underpowered to detect even large differences in risk by genotype and that there was evidence of heterogeneity between studies ($p=0.02$; $I^2 = 62\%$).
484. A subsequent meta-analysis on VDR polymorphisms (FokI, TaqI, Apal and BsmI) and TB susceptibility (Gao *et al*, 2010) included 23 studies. Findings were heterogeneous, which the authors suggested could partly be explained by differences between populations. Among Asian populations, there was a positive association with the FokI ff genotype (OR, 2.0; 95% CI, 1.3-3.2), a significant inverse association for the BsmI bb genotype (OR, 0.5, 95%CI 0.4-0.8) and marginal significant associations for TaqI and Apal polymorphisms. However there were no significant associations between any of the polymorphisms and TB among African or South American populations.

⁵² Standard method of determining whether a person is infected with *M tuberculosis*.

Respiratory tract infections

485. Respiratory tract infections (RTIs) refer to any infection of the sinuses, throat, airways or lungs. They are further classified as: upper respiratory tract infections (URTIs) which affect the nose, sinuses and throat; and lower respiratory tract infections (LRTIs) which affect the airways and lungs. URTIs include tonsillitis, laryngitis and the common cold. LRTIs include bronchitis and pneumonia. Influenza affects both the upper and lower respiratory tracts.

Intervention studies

486. A systematic review and meta-analysis of 11 RCTs (n=5660; mean age, 16 y) for prevention of RTIs (Bergman *et al*, 2013), reported that vitamin D supplementation significantly reduced the risk of RTI (OR, 0.64; 95% CI, 0.49-0.84; p=0.001) however there was evidence of significant heterogeneity between studies (p<0.0001; $I^2=72%$) and evidence of publication bias. The protective effect was significant in studies with daily vitamin D supplementation (OR, 0.51; 95% CI, 0.39-0.67) but not in studies which administered vitamin D in bolus doses once per month or less (OR, 0.86; 95% CI, 0.60-1.20). No effect of baseline 25(OH)D concentration on supplementation outcome was found..
487. Another systematic review (Jolliffe *et al*, 2013) of 39 studies (14 RCTs, 13 cohort, 8 case-control and 4 cross-sectional studies) reported that although associations between low serum 25(OH)D concentration and increased risk of both upper and lower RTIs were broadly consistent in observational studies, this was not supported by results from RCTs which were conflicting. Seven trials reported a protective effect of vitamin D supplementation against acute RTIs, 6 reported null effects and 1 reported adverse effects of vitamin D supplementation on risk of pneumonia recurrence.
488. Rees *et al* (2013) tested whether daily vitamin D₃ supplementation (25 µg/1000 IU) and/or calcium (1200 mg/d) reduced winter episodes and duration of URTI in a substudy (n=759) of a larger RCT of colorectal adenoma chemoprevention (n=2259; age, 45-75 years; 3-5 years duration) in the US. Supplementation did not significantly reduce winter episodes of URTI (rate ratio, 0.93; 95% CI, 0.79-1.09) including colds (RR, 0.93; 95% CI, 0.78-1.10) or influenza-like illness (RR, 0.95; 95% CI, 0.62-1.46). Supplementation also had no effect on participants with the lowest baseline serum 25(OH)D concentrations.

Observational studies

489. The systematic review by Jolliffe *et al* (2013) (see paragraph 487), of 13 cohort studies, reported positive associations between serum 25(OH)D concentration and risk of RTIs in 7 studies, no association in 3 studies, a negative association between serum 25(OH)D concentration in late pregnancy and increased risk of LRTI in 1 study; a protective effect of serum 1,25(OH)₂D or administration of alfacalcidol or calcitriol in 2 studies.
490. In a Korean birth cohort (Shin *et al*, 2013), cord 25(OH)D concentration < 25 compared to ≥ 75 nmol/L in newborns (n=525) was associated with risk of developing acute nasopharyngitis (OR, 3.41; 95% CI, 1.57-7.42; p=0.0008) during the first 6 months of life.
491. Another prospective study in Norway (Magnus *et al*, 2013) examined associations between plasma 25(OH)D concentration at 18 weeks gestation and frequency of LRTIs by 36 months (n=1248) and current asthma at 36 months (489 cases, 1183 controls). Higher maternal plasma 25(OH)D concentration at 18 weeks gestation was associated with a reduced risk of ≥ 3 vs 0 LRTIs by 36 months (RR, 0.74; 95% CI, 0.58-0.93) per 20 nmol/L increase. There was no association with asthma at 36 months.

492. A cohort study of children and adolescents (n=743; age, 7-13 years) in Canada (Science *et al*, 2013) reported an increased risk of viral RTI with 25(OH)D concentration < 50 nmol/L (HR, 1.67; 95% CI, 1.16-2.40; p=0.006) and < 75 nmol/L (HR, 1.51; 95% CI, 1.10-2.07; p=0.011)
493. A prospective population based cohort study of adults in Finland (n=1421; age, 53-73 years) investigated the association between serum 25(OH)D concentration and risk of incident hospitalised pneumonia (Aregbesola *et al*, 2013). Compared to adults in the highest tertile of serum 25(OH)D concentration (50.8-112.8 nmol/L) those in the lowest tertile (8.9-33.8 nmol/L) had a higher risk of developing pneumonia (RR, 2.6; 95% CI, 1.4-5.0; p=0.005).
494. A retrospective cohort study in Denver (Jovanovich *et al*, 2014) compared patients (n=132; age, 60±17 y) hospitalised with community acquired pneumonia (CAP) and controls (admitted to hospital within the same period and matched for age, sex, race & season) in relation to serum 25(OH)D concentration (measured 3-15 months prior to hospital admission). Serum 25(OH)D concentration < 37 nmol/L vs ≥ 37 nmol/L was associated with increased odds of CAP (OR, 2.57; 1.08-6.08; p=0.03).

Chronic obstructive pulmonary disease (COPD)

495. Chronic obstructive pulmonary disease (COPD) refers to a group of lung diseases that obstruct airflow, making breathing difficult. No studies could be identified of vitamin D and risk of COPD. All the identified studies examined the relationship between serum 25(OH)D concentration and COPD in individuals with pre-existing disease; the findings are, therefore, not applicable to the general population.

Summary – Infectious disease

496. The majority of evidence in this area relates to use of vitamin D as a therapeutic agent in patients with pre-existing disease and whether vitamin D can reduce severity or progression of the disease. Findings from such studies are not applicable to the general population.
497. Evidence on vitamin D and infection is inconsistent and mainly observational. RCTs do not generally show a beneficial effect of vitamin D supplementation on autoimmune disease risk.
498. No RCTs on the effect of vitamin D supplementation for prevention of TB could be identified. Observational studies report a positive association between serum 25(OH)D concentration and TB risk. Studies examining associations between VDR gene polymorphisms and susceptibility to TB are inconclusive.
499. Out of 2 systematic reviews/meta-analyses of RCTs on the effect of vitamin D supplementation on RTIs, 1 reported beneficial effects of vitamin D supplementation in reducing RTI risk while the other reported conflicting results. Findings from cohort studies are generally supportive of an inverse association between serum 25(OH)D concentration and RTIs with serum 25(OH)D concentrations ranging between < 25 and < 50 nmol/L associated with increased risk for developing RTIs.
500. No studies could be identified on vitamin D and risk of developing COPD.

Neuropsychological functioning (*cognitive function, dementia, autism, depression, schizophrenia*)

501. The effect of vitamin D on brain function is an area of growing interest but, for many conditions, the evidence base is currently limited. From a biological perspective, vitamin D receptors and 1- α hydroxylase have been identified in the cerebral cortex and cerebellum suggesting that the brain may synthesise 1,25(OH)₂D to regulate local functions. Animals deprived of vitamin D early in development show evidence of abnormal brain development. This raises the possibility that vitamin D might impact on various aspects of brain function (such as mood or cognition) or diseases caused by abnormal brain function (such as autism and schizophrenia).

IOM Report

502. The IOM considered the effect of vitamin D on cognition & dementia, autism, depression, schizophrenia. The report noted that the evidence base comprised observational data mostly from cross-sectional studies with shortcomings in study design and quality.

Cognition and dementia

Intervention studies

503. Two small studies examined the effects of vitamin D supplements on cognition in adults over a few weeks (Przybelski *et al*, 2008; Stein *et al*, 2011). Both had significant design weaknesses. In an unblinded study (Przybelski *et al*, 2008) of nursing home residents (n=63; mean age, 87 y), participants were supplemented with vitamin D₂ (1250 μ g/50000 IU) 3 times/week for 4 weeks if their serum 25(OH)D concentration was < 62 nmol/L; participants in the comparison group (with 25(OH)D concentration > 62 nmol/L) were not given a placebo. Vitamin D₂ supplementation had no effect on cognition.

504. Stein *et al* (2011) examined the effect of high dose vitamin D on memory and disability on community dwelling individuals with mild/moderate Alzheimer's disease (n=63; age > 60 y). All participants were supplemented daily with vitamin D₂ (25 μ g/1000 IU) throughout the trial. After 8 weeks run-in, participants were randomised to receive placebo or a high dose vitamin D₂ supplement (300 μ g/12,000 IU per capsule) for 8 weeks, initially receiving 2 capsules, 3 times/day which was subsequently reduced to 0-2 capsules/day to maintain serum 25(OH)D concentrations at 130-175 nmol/L at 2, 4 and 6 weeks. High-dose vitamin D did not provide any benefit for cognition over low-dose vitamin D.

505. Rossom *et al* (2012) conducted a *post hoc* analysis of cognition related outcomes of women (n=4,143; aged \geq 65 y) participating in the Women's Health Initiative (WHI) Calcium and vitamin D trial and the WHI Memory Study. Participants in the trial were randomised to receive vitamin D₃ (10 μ g/400 IU/d) and calcium carbonate (1000 mg/d) or placebo. During a mean follow-up of 7.8 years, vitamin D and calcium supplementation had no effect on cognitive decline or incident dementia.

Observational studies

506. A systematic review of observational studies (van der Schaft *et al*, 2013) assessed the association between serum 25(OH)D concentration and cognitive function in 25 cross-sectional (n=48,680) and 6 prospective studies (n=10,896). The majority of prospective studies reported a significant decline in 1 or more cognitive function tests or a higher frequency of dementia in participants with lower compared to higher serum 25(OH)D concentration. The cross-sectional studies found a significantly worse outcome on one or more cognitive function tests or higher frequency of dementia associated with lower serum 25(OH)D concentration. Nine of the cross-sectional studies included the mean serum 25(OH)D concentration of participants (45-76 nmol/L).

Depression

507. Depression is recognised to be more common during the winter at Northern Latitudes when serum 25(OH)D concentrations are lowest.

RCTs

508. A systematic review and meta-analysis (Spedding, 2014) identified 15 RCTs on the effect of vitamin D in depression. The authors reported wide variation in study methodology and diversity in study populations. Many of the studies were in patients and 6 RCTs did not report baseline serum 25(OH)D concentration. Vitamin D supplemental doses varied from 10-460 µg/d (400-18,400 IU) across the 15 trials. Eight out of the 15 studies were classified as having flawed study designs that limited their ability to demonstrate a change in the serum 25(OH)D concentration of the intervention group⁵³. Of the 7 studies considered to be without flawed study design, 6 showed an improvement in depression with vitamin D supplementation; 6 of the 9 flawed studies had a null result. Only 2 studies were included in a meta-analysis of studies without flaws as they used the same depression measure⁵⁴ which showed a significant improvement in depression (0.78; 95% CI, 0.24-1.27). Two studies were also included in the meta-analysis of flawed studies (due to the diverse outcome variables used in the other studies) which showed a significant negative effect of vitamin D supplementation (-1.1; 95% CI, -0.7 to -1.5).
509. Seasonal affective disorder (SAD) is a condition characterised by symptoms of depression, anxiety, irritability, appetite changes, hypersomnia and fatigue that occur during winter months and abate in the spring and summer (Rosenthal *et al*, 1984). Women are affected more than men and the average age at onset is comparable to that of major depression. Some evidence suggests that incidence increases with latitude and therefore reduced sun exposure, and phototherapy with broad spectrum bright artificial light (>2500 lux) may improve symptoms within days in some patients. Several small trials have failed to show any consistent beneficial effect of vitamin D supplementation in this context (reviewed in Bertone-Johnson, 2009).

Observational studies

510. Cross-sectional data on serum 25(OH)D concentration and depression are inconsistent but tend to show that patients with major or minor degrees of depression have lower serum 25(OH)D concentration than individuals without depression (Hoogendijk 2008; Ganji *et al*, 2010; Hoang *et al*, 2011). However, depression could alter diet or behaviour in ways which would reduce serum 25(OH)D concentration.
511. A systematic review and meta-analysis of observational studies (10 cross-sectional, 1 case-control, 3 cohort) reported that: in the case control study, lower serum 25(OH)D concentration was found in people with depression compared with controls; in cross-sectional studies, there was an increased OR for lowest vs highest serum 25(OH)D serum concentration (OR=1.31; 95% CI, 1.0-1.71); the cohort studies showed a significantly increased hazard ratio of depression for the lowest vs highest categories of serum 25(OH)D concentration (HR=2.21; 95% CI, 1.4-3.49).

Autism

512. It has been proposed that low serum 25(OH)D concentration *in utero* or in early postnatal life may be an environmental risk factor for autism (Grant & Soles, 2009). The evidence in support of this is very limited and is mainly ecological. There is no applicable evidence available from intervention trials or from significant case-control or prospective studies.

⁵³ Interventions that reduced 25(OH)D concentration in the intervention group, interventions that did not significantly improve 25(OH)D concentration, studies that did not measure baseline 25(OH)D concentrations and studies which included participants whose baseline 25(OH)D concentrations indicated 'sufficiency'.

⁵⁴ Beck Depression Inventory

Schizophrenia

Intervention studies

513. No evidence is available from intervention trials in relation to vitamin D supplementation and subsequent development of schizophrenia.

Observational studies

514. Epidemiological studies have reported a tendency for people with schizophrenia to be born in winter (Moskovitz, 1978). It has been suggested that this might be related to a decrease in maternal serum 25(OH)D concentration during the winter months leading to low prenatal serum 25(OH)D concentration which may predispose to schizophrenia (McGrath, 1999). It has been proposed that this might explain epidemiological findings relating to schizophrenia including the impact of season of birth, latitude gradients in incidence and prevalence, the increased risk in dark-skinned migrants to certain countries, and the urban-rural gradient (McGrath et al 2010a).
515. A large case control study in Denmark (n=848), which investigated the association between neonatal serum 25(OH)D concentration and subsequent risk of schizophrenia, reported that both high and low serum 25(OH)D concentration in neonates were associated with increased risk of developing schizophrenia in later life (McGrath 2010b). Serum 25(OH)D concentrations were measured from dried neonatal blood samples and divided into quintiles: <19.7, 19.7-30.9, 31-40.4, 40.5-50.9 and > 51 nmol/L. Compared with the 4th quintile, neonates in the lowest quintile (<19.7 nmol/L) were at increased risk of developing schizophrenia (RR, 2.1; 95% CI, 1.3-3.5). Neonates in the 2nd (19.7-30.9 nmol/L) and 3rd quintiles (31-40.4 nmol/L) were also at increased risk of developing schizophrenia (RR, 2.0; 95% CI, 1.3-3.2 and RR, 2.1; 95% CI, 1.3-3.4 respectively) as were neonates in the highest quintile (>51 nmol/L) (RR, 1.7; 95% CI, 1.04-2.8).
516. A systematic review and meta-analysis of 19 observational studies (8 cross-sectional, 10 case-control; 1 nested case-control) with measures of serum 25(OH)D concentrations in schizophrenic patients (Valipour *et al*, 2014) reported that the overall mean difference in serum 25(OH)D concentration between schizophrenic and control participants was about -15 nmol/L (95% CI, -27, -3 nmol/L). The overall prevalence of vitamin D deficiency (defined as 25(OH)D concentration < 50 nmol/L) in schizophrenic patients was 65% (95% CI, 46-84%); however there was considerable between study heterogeneity ($I^2 = 84.8$). Meta-analysis of the odds ratios reported in studies found that individuals with serum 25(OH)D concentration < 50 nmol/L compared with those > 50 nmol/L were more likely to have schizophrenia (OR, 2.16; 95% CI, 1.32-3.56).

Summary – Neuropsychological functioning

517. Evidence linking vitamin D to cognition and to depression is supported mainly by cross-sectional data which report an association between lower 25(OH)D concentration and poor cognitive function. This finding might be due to reverse causation since changes in cognition and depression may alter diet and/or behaviour in a way which would reduce serum 25(OH)D concentration. Beneficial effects of vitamin D on cognition or depression are not currently supported by robust clinical trials.
518. Evidence relating vitamin to autism is very limited and mainly ecological.
519. No intervention trials have examined the relationship between vitamin D and schizophrenia. Evidence linking vitamin D to schizophrenia is mainly ecological. Cross-sectional and case-control studies report that serum 25(OH)D concentration < 50 nmol/L is associated with increased schizophrenia risk; however, 1 case-control study found that serum 25(OH)D concentration < 20 nmol/L and > 50 nmol/L is associated with increased schizophrenia risk.

Oral health

520. Vitamin D can impact on oral health by interference in mineralisation of teeth and by modifying the rate of progression of bone loss during periodontal disease. The impact of vitamin D deficiency on tooth development has been recognised for many years (Dick, 1916) and has been described in both vitamin D dependent rickets (Zambrano *et al*, 2003; Kikuchi *et al*, 1988) and in hypophosphataemic vitamin D resistant rickets (Nishino *et al*, 1990; Goodman *et al*, 1998; Seow *et al*, 1995; Muryama *et al*, 2000). Teeth are relatively protected during the mineralisation phase so effects on teeth are fewer than those seen skeletally. However there are disturbances of both enamel and dentine formation, that are very similar in the various conditions. The enamel that develops is hypoplastic, pitted and relatively thin with reduced mineralisation making the teeth more susceptible to caries. The dentine is abnormal in macroscopic structure and also has lower than normal levels of mineralisation. Individuals with rickets develop high levels of dental caries and tooth wear that spread rapidly through the enamel and underlying thinned dentine to expose the dental pulp, which results in early pulp death. There is also increased susceptibility to periodontal disease or periodontitis, an inflammatory disease of the attachment apparatus of the tooth to the gum (the periodontium) comprising the gum, the supporting periodontal ligament and the alveolar bone.
520. A bacterial biofilm (dental plaque) forms on the surface of teeth throughout their life. The bacteria in mature plaque produce metabolites that irritate the gingival tissues around the margin of the tooth causing a localised inflammatory response or gingivitis. This initial lesion can remain confined to the gingiva or it can progress to the periodontium resulting in periodontitis. This is characterised by progressive loss of attachment of the tooth to its supporting bone. The bone destruction is thought to be a consequence of the host inflammatory response removing bone in an inflamed environment. The bone does not regrow and loss of support is permanent. The variables responsible for progression from gingivitis to periodontitis are presently unclear. Vitamin D could affect periodontal disease by modifying the rate of progression of bone loss or through anti-inflammatory effects.

IOM Report

521. The IOM did not consider vitamin D in relation to oral health.

Evidence considered

RCTs

522. No RCTs on vitamin D and oral health could be identified.

Observational studies

523. Cross sectional studies have reported an association between higher serum 25(OH)D concentration and reduced risk of gingival inflammation (Dietrich *et al*, 2005), periodontitis (Millen *et al*, 2013) and tooth loss (Jimenez *et al*, 2014). A large cross-sectional analysis of NHANES data (n=11,202, age ≥ 20 y) reported a significant and inverse association between serum 25(OH)D concentration and periodontal tooth loss in men and women aged ≥ 50 y (Dietrich *et al*, 2004); however no association was found between BMD of the total femoral region and tooth loss (Dietrich *et al*, 2004).
524. A prospective cohort study in Germany, with 5 years follow-up (n=1,904; age, 20-79 y), reported that higher serum 25(OH)D concentration at baseline was associated with a lower risk of tooth loss (Zhan *et al*, 2014). Compared with participants in the 1st quintile of serum 25(OH)D concentration (mean, 12.5 nmol/L), those in the 5th quintile (mean, 67.6 nmol/L) had a 23% lower risk of tooth loss (RR: 0.77; 95% CI: 0.60-0.99). Since serum 25(OH)D concentration was only measured once at baseline information was not available on whether concentrations changed during the follow-up period.

Genetic studies

525. It has been suggested that associations between vitamin D and periodontal disease progression may be independent of its role in terms of bone metabolism and relate more to the role of VDR receptors in regulating inflammatory disease (Dietrich *et al*, 2004; Amano *et al*, 2009). A number of studies have linked specific VDR gene polymorphisms with aggressive forms of periodontal disease (Brett *et al*, 2005; Deng *et al*, 2011; Hennig *et al*, 1999; Meng *et al*, 2000; Park *et al*, 2006; Sun *et al*, 2002; Yoshihara *et al*, 2001) as well as with chronic adult disease (de Brito *et al*, 2004; Martelli *et al*, 2011; Tachi *et al*, 2003). These studies suggest that the relationship between periodontal attachment loss and serum 25(OH)D concentration is moderated by alterations in host immunity rather than a direct impact on calcium metabolism and bone turnover in some members of the population.

Summary – Oral health

526. Evidence from RCTs on effects of vitamin D supplementation on oral health is lacking.

527. Cross-sectional data show a positive association between serum 25(OH)D concentration and measures of oral health. One cohort study found an inverse association between serum 25(OH)D concentration and tooth loss.

528. Evidence from genetic studies suggests that associations between vitamin D and periodontal disease is influenced by changes in host immunity rather than through effects on calcium metabolism.

Age-related macular degeneration

530. Age-related macular degeneration (AMD) is a progressive chronic disease resulting in damage to the central retina and is a major cause of visual impairment in older people. In the dry or atrophic form of the condition, the retinal pigment epithelium degenerates leading to the development of drusen⁵⁵. The wet or exudative form of AMD is characterised by new blood vessel formation under the macular region of the central retina, which results in plasma leakage, retinal haemorrhage, inflammation and scarring. Risk factors for the development of AMD include advancing age, family history, race, genetic mutations, sunlight exposure, hypertension, high dietary fat, obesity and smoking (Lim *et al*, 2012).
531. The pathogenesis of AMD is not clearly understood but angiogenesis is thought to play a role (Rosenfeld *et al*, 2006); inflammation and immunological changes are also implicated (Zarbin, 2004; Anderson *et al*, 2010). The potential role of vitamin D in the pathogenesis of AMD has been investigated because of its inhibitory actions on angiogenesis (Mantell *et al*, 2000) and studies suggesting an association between vitamin D and inflammation and immune function (see section x).

IOM report

532. The IOM report did not consider age-related macular degeneration.

Observational studies

533. A protective association was found between serum 25(OH)D concentration and prevalence of early (but not advanced) AMD in a nationally representative sample of adults in the US (n=7,752; aged ≥ 40 years) (Parekh *et al*, 2007). The odds ratio for early AMD in adults in the highest (> 85 nmol/L) vs lowest (< 42 nmol/L) quintile of serum 25(OH)D concentration was 0.64 (95% CI, 0.5-0.8; p trend <.001). Another cross-sectional analysis in Israel (Golan *et al*, 2011) found no significant difference in serum 25(OH)D concentration between those with (n= 1,045; mean age, 77.66±7) and without (n=8124; mean age, 76 y AMD).
534. Millen *et al* (2011) investigated the relationship between serum 25(OH)D concentration and prevalence of early AMD in postmenopausal women (n=1313; age, 50-79 y) participating in the Carotenoids in Age-Related Eye Disease Study in the USA. Serum 25(OH)D concentration was measured at baseline and AMD status was assessed from fundus photographs after 6 years. In multivariate models, no significant relationship was observed between serum 25(OH)D concentration and early or advanced AMD but there was a significant age interaction (p=0.0025). Serum 25(OH)D concentration (highest vs lowest⁵⁶ quintile) was significantly associated with a lower risk of early AMD in women < 75 y but an increased risk in older women; however this association was no longer significant after further adjustment for BMI and physical activity.
535. A systems biology-based analysis investigated the role of vitamin D metabolism in the pathogenesis of AMD in a cohort of sibling pairs (n=481) discordant for AMD (Morrison *et al*, 2011). After adjustment for established risk factors for AMD, including genetic polymorphisms and smoking, UV irradiation was associated with a lower risk of AMD (p=0.001). Serum 25(OH)D concentration (measured in 50 sibling pairs) was lower in individuals with AMD than in their unaffected siblings, but this was not statistically significant. A candidate gene approach was used to examine variation in key genes regulating vitamin D metabolism (including those encoding VDR, CYP27B1, CYP24A1 and CYP27A) in participants (n=2,525) comprising the sibling pairs and their extended families, individuals from a separate case-control study from Greece and a prospective nested case-control population from the Nurse's Health Study and Health Professionals Follow-Up Study in the US. Single point variants in the CYP24A1 gene were shown to

⁵⁵ Focal deposits of extracellular debris under the retina. The largest single component of drusen is lipid.

⁵⁶ Median (range): Q1 = 30 (7, 38) nmol/L; Q5 = 85 (>75, 165) nmol/L

influence the risk of AMD after adjusting for age, sex and smoking, in all the populations separately and in a meta-analysis.

Summary - Age-related macular degeneration

536. No intervention studies on vitamin D supplementation and AMD could be identified.

537. Evidence on serum 25(OH)D concentration and AMD is mainly from cross-sectional studies which are inconsistent. One small study reported that variation in the CYP24A1 gene may play a role in the pathogenesis of AMD

Conclusions – non-musculoskeletal health outcomes

538. There is no evidence from intervention studies showing that vitamin D supplementation in pregnancy improves the woman's own reproductive health or pregnancy outcome. The observational evidence is mixed. Maternal vitamin D supplementation during pregnancy has beneficial effects in reducing the risk of neonatal hypocalcemia but there is little evidence from intervention studies or observational evidence to indicate any additional benefits for the baby. The contribution made by vitamin D supplementation in pregnancy to the later serum 25(OH)D concentration of the unsupplemented, exclusively breastfed baby is unclear. Data from a cross-sectional study suggest that the mean serum 25(OH)D concentration of an exclusively breastfed infant in the UK is unlikely to be maintained > 25 nmol/L during winter without vitamin D supplementation.
539. Evidence from observational studies suggests an inverse association between serum 25(OH)D concentration and colorectal cancer risk. Since these studies might be confounded by other factors that affect cancer risk they do not provide compelling evidence of a protective effect of vitamin D on colorectal cancer risk. Observational studies on other cancers do not suggest an association with serum 25(OH)D concentration.
540. Although observational data from population cohort studies suggest a protective effect of increasing serum 25(OH)D concentration on risk of CVD and hypertension, this is not supported by results from intervention trials.
541. Evidence from intervention studies indicate that vitamin D has no effect on mortality risk. Although observational data suggest an inverse association between serum 25(OH)D concentration and mortality risk this might also be due to reverse causality or confounding by other factors associated with mortality such as obesity, physical activity and smoking.
542. There is a paucity of data on the effect of vitamin D supplementation on autoimmune disease. Evidence from observational studies is inconsistent and may also be confounded by other factors that affect autoimmune disease. The data are insufficient to draw firm conclusions.
543. Evidence on vitamin D and infectious disease is mainly observational. The observational data suggest an inverse association between serum 25(OH)D concentration and infectious disease risk but these studies are difficult to interpret since it is unclear if low serum 25(OH)D concentration is a cause or consequence of the infection. The evidence is insufficient to draw any firm conclusions.
544. Data on vitamin D and neuropsychological functioning is mainly observational and insufficient to draw conclusions.
545. Evidence linking vitamin D to cognition and depression is supported mainly by cross-sectional data. However, the findings might be due to reverse causation since changes in cognition or depression may alter diet and/or behaviour in a way which would reduce serum 25(OH)D concentration. Beneficial effects of vitamin D on cognition or depression are not currently supported by robust clinical trials.
546. Evidence on the relationship between serum 25(OH)D concentration and oral health is mainly observational and little information is available on the serum 25(OH)D concentration associated with poor oral health outcomes. There is insufficient evidence on vitamin D and oral health to draw firm conclusions.
547. There are insufficient data to draw conclusions on the relationship between serum 25(OH)D concentration and AMD.

Selection of health outcomes to inform the setting of DRVs for vitamin D

548. Evidence for a relationship between vitamin D and a range of musculoskeletal and non musculo-skeletal health outcomes was reviewed in order to assess whether any might be used to inform the setting of DRVs for vitamin D. The health outcomes examined were those considered to be of public health importance.
549. The evidence on vitamin D and musculoskeletal health was considered to be suggestive of beneficial effects of vitamin D on:
- a. rickets in infants and children;
 - b. osteomalacia in all adult age groups;
 - c. falling in adults > 50 years;
 - d. muscle strength and function in young people and adults.
550. These musculoskeletal health outcomes were therefore selected as the basis for setting the DRVs for vitamin D.
551. Data on vitamin D and any non-musculoskeletal health outcome were considered to be insufficient at this time to inform the setting of DRVs for vitamin D.
552. Since serum 25(OH)D concentration reflects exposure to vitamin D from both sunlight and diet, the next step was to determine if it was possible to identify a distribution of serum 25(OH)D requirements for the selected musculoskeletal health outcomes or, if this was not possible, a threshold serum 25(OH)D concentration below which the risk of these musculoskeletal health outcomes is increased. The current lower limit used to indicate increased risk of deficiency is a serum 25(OH)D threshold concentration of 25 nmol/L (DH, 1998). Concentrations below this are associated with increased risk of rickets and osteomalacia.
553. Serum 25(OH)D concentrations in the studies on musculoskeletal health outcomes judged to be suggestive of beneficial effects of vitamin D (rickets, osteomalacia, falls, muscle strength & function) were considered further to assess whether a distribution or threshold serum 25(OH)D concentration associated with beneficial effects could be identified.
- *Rickets* - Rickets was present at individual and mean serum 25(OH)D concentrations < 25 nmol/L in the majority of studies considered.
 - *Osteomalacia* – Based on the limited evidence (mainly case reports) individual serum 25(OH)D concentrations were < 20 nmol/L in case reports. In 2 cross-sectional studies, mean concentration was < 15 nmol/L in one and individual concentrations were < 7.5 nmol/L in the other.
 - *Falls* – The evidence is mixed but, on balance, is suggestive of beneficial effects of vitamin D supplementation in reducing fall risk in adults > 50 years with mean baseline serum 25(OH)D concentrations over a range of values. There is also evidence of an adverse effect of an annual high dose (12,500 µg/500,000 IU)
 - *Muscle strength and function* - Overall, the evidence suggests that vitamin D supplementation may improve muscle function in adolescents and adults < 50 years with a mean serum 25(OH)D concentration < 30 nmol/L and in adults > 50 years with mean baseline serum 25(OH)D concentrations over a range of values.

554. With the exception of case reports, the studies considered only provided mean/median serum 25(OH)D concentrations of participants. It was not possible, therefore, to establish a distribution of serum 25(OH)D concentrations associated with the selected musculoskeletal health outcomes.
555. Overall, there was wide variability in the mean and individual serum 25(OH)D concentrations associated with increased risk of rickets, osteomalacia, falls and improvement in muscle strength and function together with many uncertainties in the data, including the use of predefined cutoffs; however, risk appears to increase at serum 25(OH)D concentrations below 20-30 nmol/L.
556. An additional complexity in the interpretation of the data is the high inter-assay and inter-laboratory variation in serum 25(OH)D concentration measurements (see chapter 4). Since a range of assay methods were utilised to measure serum 25(OH)D concentration in the different studies on musculoskeletal health, it is difficult to make comparisons between studies on the serum 25(OH)D concentration associated with increased risk. Since the data do not allow differentiation between a serum 25(OH)D threshold concentration of 20 vs 25 vs 30 nmol/L, the current threshold of 25 nmol/L (DH, 1998) is retained. This threshold serum 25(OH)D concentration is not diagnostic of disease but indicative of increased risk of poor musculoskeletal health.
557. A serum 25(OH)D concentration of 25 nmol/L indicates the concentration below which the risk of poor musculoskeletal health is increased at a population level and therefore represents a '*population protective*' concentration. It does not refer to the mean target serum 25(OH)D concentration for a particular life-stage group but rather the serum 25(OH)D concentration that the majority (97.5%) of individuals should reach or be above (all year round) in terms of protecting musculoskeletal health.
558. Since it was not possible to identify the serum 25(OH)D concentration below which risk of poor musculoskeletal health is increased during pregnancy and lactation because of insufficient or inadequate evidence, the '*population protective*' concentration of 25 nmol/L was extended to these groups.
559. A serum 25(OH)D concentration of 25 nmol/L is therefore selected as the basis for establishing the Reference Nutrient Intake for vitamin D; i.e., the mean vitamin D intake required to achieve a serum 25(OH)D concentration \geq 25 nmol/L, throughout the year, by the majority (97.5%) of the population. The mean vitamin D intake refers to the mean or average intake over a period of time (e.g., one week) and takes account of day to day variations in vitamin D intake.
560. The vitamin D intake and the summer sunshine exposure required to achieve a serum 25(OH)D target concentration of \geq 25 nmol/L is considered in chapter 9.

7. Potential adverse effects of high vitamin D intake/high serum 25(OH)D concentration

Vitamin D toxicity

561. Cutaneous synthesis of vitamin D is regulated so that prolonged sunshine exposure does not lead to excess production (see paragraph 23). Excessive vitamin D intakes have, however, been shown to have toxic effects (Vieth *et al*, 2006). Vitamin D toxicity results in hypercalcaemia (elevated serum calcium) caused by increased intestinal calcium absorption and mobilisation of calcium from the bone (Jones, 2008). Hypercalcaemia can result in deposition of calcium in soft tissues, diffuse demineralisation of bones, and irreversible renal and cardiovascular toxicity.
562. Other adverse effects that have been linked with high vitamin D intakes or high serum 25(OH)D concentration include an increased incidence of falls and fractures, increased rates of pancreatic and prostatic cancer and increased total mortality (i.e., from all causes combined). Evidence for these associations is less robust and consistent than that relating to hypercalcaemia.

Supplemental sources of vitamin D

563. Food supplements containing up to 250 µg (10,000 IU) of vitamin D per daily dose are available. Most multi-vitamin food supplements contain 5 µg (200 IU) of vitamin D per daily dose.

Recommended upper intake levels for vitamin D

UK

564. In 2003, the Expert Group on Vitamins and Minerals (EVM) reported on the safety of vitamin and mineral supplements and recommended maximum advisable levels of intake. Safe Upper Levels (SULs) were established when supported by sufficient data. The SUL represents an intake that can be consumed daily over a lifetime without significant risk to health. A Guidance Level (GL) was set when the evidence base was inadequate to establish an SUL. GLs represent an approximate indication of intakes that would not be expected to cause adverse effects; they are less secure than SULs because they are derived from limited data.
565. The EVM found insufficient data to establish an SUL for vitamin D. Based on data relating to hypercalcaemia, a GL for supplemental vitamin D intake (i.e., in addition to dietary intake) of 25 µg/d was set for adults. Scaling on a body weight basis to children and infants was not considered appropriate because of concerns that it might lead to the recommended intake for an infant not being met.

USA

566. In its 2011 review of the evidence on vitamin D and health outcomes, the IOM selected onset of hypercalcaemia and related toxicity as the basis for establishing a Tolerable Upper Intake Level (UL) for all age groups except infants (0-12 months). Retarded linear growth was used as the basis for establishing the UL for infants. The UL is defined as the highest average daily intake of a nutrient that is likely to pose no risk of adverse health effects to nearly all persons in the population. It applies to intakes on a chronic basis among free-living persons.
567. A UL of 100 µg/d (4,000 IU) was set for adults ≥ 19 y of age. A UL of 25 µg/d (1,000 IU) was set for infants 0-6 months and 38 µg/d (1,520 IU) for infants aged 6-12 months. The UL for pregnant and lactating women was the same as that for adults.
568. The IOM noted the paucity of long term studies with vitamin D intakes > 250 µg/d (10,000 IU) or where serum 25(OH)D concentrations above 250 nmol/L were observed but, based on the available data, considered it unlikely that symptoms of toxicity would be observed at vitamin D intakes below 250 µg/day.

Europe

569. In 2012, the European Food Safety Authority (EFSA) revised the Tolerable Upper Levels (UL⁵⁷) for all age groups. A UL is intended to apply to all groups of the general population, including more sensitive individuals, but with the exception in some cases of discrete, identifiable sub-populations who may be especially vulnerable to one or more adverse effects (e.g. those with unusual genetic predisposition, certain diseases, or receiving the nutrient under medical supervision).
570. For adults, hypercalcaemia was selected as the indicator of toxicity and the UL was set at 100 µg/d (4,000 IU), including for pregnant and lactating women. A UL of 100 µg/d (4,000 IU) was also set for children and adults aged 11-17 y because, owing to phases of rapid bone formation and growth, it was considered unlikely that this age group would have a lower tolerance for vitamin D compared to adults. A UL of 50 µg/d (2,000 IU) was set for children aged 1-10 y to take account of their smaller body size. For infants (0-12 months), the previous UL of 25 µg/d (1,000 IU), based on data relating high vitamin D intakes to impaired growth and hypercalcaemia, was retained.

Committee on Toxicity of chemicals in food, consumer products and the environment (COT)⁵⁸

571. For the purposes of the current review, the COT was requested to review the data on potential harmful effects of high vitamin D intakes, either regularly over prolonged periods, or as single or occasional large doses and to also consider whether some groups of people might be particularly vulnerable to high intakes of vitamin D.
572. The EFSA and IOM reviews were used as the initial bibliographic sources for the COT's review of the evidence with an updated and expanded literature search.
573. The findings and conclusions of the COT are summarised below. COT's full statement with its detailed considerations can be found on its website (<http://cot.food.gov.uk/sites/default/files/VitaminDstatement.pdf>).

Review of the evidence on adverse effects

Adults

Hypercalcaemia

574. Total calcium in the blood and extracellular fluid, is maintained at a concentration of approximately 2.5 mmol/L (range 2.25-2.6 mmol/L) and ionised calcium at 1.1-1.4 mmol/L (EFSA, 2012). Hypercalcaemia is generally defined as a total calcium concentration greater than 2.75 mmol/L. If serum calcium increases above 3 mmol/L, the ability of the kidney to reabsorb calcium is exceeded and hypercalciuria can follow. Hypercalciuria is defined as urinary calcium excretion > 250 mg/d in women and 275-300 mg/d in men.
575. A number of case reports of vitamin D intoxication following high medicinal doses or excessive use of food supplements, have been reported in the literature. In these case reports, serum 25(OH)D concentrations of 300 to more than 1000 nmol/L were associated with intoxication. However, case reports provide limited information for risk assessment purposes because the doses consumed, where known, have varied in amount and duration.
576. Adverse effects have also been reported in intervention studies examining the effect of vitamin D supplementation on various health outcomes. These studies provide information on population groups as well as supplemental doses/serum 25(OH)D concentrations associated with reported adverse effects.

⁵⁷ Definition of UL equivalent to that used by the IOM.

⁵⁸ The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) is an independent scientific committee that provides advice to Government Departments and Agencies on matters concerning the toxicity of chemicals.

Trials varied in design and few administered vitamin D doses > 100 µg/d (4,000 IU). In most trials where higher daily doses were used, it was rarely for longer than a few months. Only isolated instances of hypercalcaemia were reported in the intervention studies. Serum calcium concentrations increased in some trials but remained within the normal range. Only two studies (Heaney *et al*, 2003; Barger-Lux *et al*, 1998) used one or more doses ≥ 100 µg/d (4,000 IU) in the absence of calcium supplements, for ≥ 2 months.

577. Heaney *et al* (2003) investigated the relationship between steady state vitamin D₃ intake and serum 25(OH)D concentration. Vitamin D₃ doses of 0, 25, 125 or 250 µg/d (10,000 IU) were administered to healthy men (n=67) for 20 weeks over the winter in Omaha, US. Mean serum 25(OH)D concentration was 70 nmol/L at baseline, which increased in proportion to the dose. Limited information was provided on changes in serum calcium concentrations but indicated that none of the men in the top two dose groups (n=31) had concentrations above the normal reference range after treatment. The IOM observed that vitamin D intakes of 125 µg/day (5,000 IU) achieved serum 25(OH)D concentrations of 100-150 nmol/L (but not exceeding 150 nmol/L) after 160 days of administration.
578. Barger-Lux *et al* (1998) investigated the relationship between graded oral dosing with vitamin D₃ for 8 weeks and changes in serum 25(OH)D concentration in healthy young men (n=116; mean age 28±4 y). Doses of 25, 250 or 1250 µg/d resulted in mean (± SEM) increases in serum 25(OH)D concentration of 28.6 (± 5.3), 146.1 (± 12.0) and 643.0 (± 42.7) nmol/L respectively above the mean baseline concentration (67 ± 25 nmol/L). No statistically significant changes were detected in mean baseline serum calcium concentration (2.41 ± 0.07 mmol/L).

Kidney stones

579. Prolonged hypercalciuria is a risk factor for kidney stones. Although available human studies suggest that high intakes of vitamin D alone are not associated with an increased risk of kidney stones, combined supplementation with calcium may increase risk. Jackson *et al* (2006) reported an increased risk of kidney stones in women given a daily calcium supplement of 1,100 mg plus 10 µg (400 IU) of vitamin D for up to 7 years. However, total intakes of vitamin D in this study were below those associated with hypercalcaemia.

Fall and fractures

580. An intervention study (Sanders *et al*, 2010) of women in Australia (n=2,256; age ≥ 70 y) reported an increased risk of fracture in the vitamin D₃ supplemented group (single annual dose of 12,500 µg/500,000 IU for 3-5 years) compared to the placebo group (IRR⁵⁹, 1.15; 95% CI, 1.02-1.30 for fractures; IRR, 1.26; 95% CI, 1.00-1.59 for falls). Serum 25(OH)D concentration, which was measured in a subsample (n=137) of participants, increased from a median of 49 nmol/L at baseline to 120 nmol/L after 1 month in the vitamin D supplemented group and 90 nmol/L at 3 months, and remained higher than concentrations in the placebo group 12 months after dosing. Data on serum levels of calcium were not reported.
581. Another study (Smith *et al*, 2007) reported an increase in non-vertebral fracture in women (but not men) given an annual intra-muscular injection of vitamin D₂ (7500 µg/300,000 IU). No effect was observed on the frequency of falls.
582. A cohort study in the USA (Cauley *et al*, 2011) reported that serum 25 (OH)D concentration ≥ 50 nmol/L was associated with lower fracture risk in white women but a higher fracture risk in black women (OR 1.45, 95%CI 1.06-1.98); serum concentrations ≥ 75 nmol/L were associated with a higher risk of fracture in Asian women.

⁵⁹ Incident rate ratio.

Cancer

Pancreatic cancer

583. Some observational studies have suggested an association between higher vitamin D intakes or serum 25(OH)D concentration and risk of pancreatic cancer, but the finding has not been consistent. Skinner *et al* (2006) and Giovannucci *et al* (2006) did not find an association while Stolzenberg-Solomon *et al* (2006) reported a 3-fold increase in pancreatic cancer risk with highest (65.5 nmol/L) vs lowest (<32.0 nmol/L) quintile of serum 25(OH)D concentration (OR, 2.92; 95% CI, 1.56-5.48, *ptrend* = 0.001). A subsequent pooled study using data from several cohorts (Stolzenberg-Solomon *et al*, 2010) reported that serum 25(OH)D concentrations ≥ 100 nmol/L compared to 50-< 75 nmol/L were associated with a statistically significant increase in risk of pancreatic cancer (OR, 2.12; 95% CI, 1.23-3.64). However, it has been suggested that the positive association was a statistical artefact arising from the choice of cut-points and that merging the top two groups largely abolished the relationship (Baggerly & Garland, 2012).

Prostate cancer

584. A nested case-control study (Tuohimaa *et al*, 2004), using stored serum from 3 cohorts of Nordic men (n=622 cases; n=1451 controls) reported that both low (≤ 19 nmol/L) and high (≥ 80 nmol/L) serum 25(OH)D concentration was associated with higher risk of prostate cancer. Another a nested case-control study in Finland (Faupel-Badger *et al*, 2007) found no association between serum 25(OH)D concentration in men who were smokers (n=296 cases, n=297 controls) and risk of prostate cancer.

All-cause mortality

585. The IOM (2011) identified 5 cohort studies (Sambrook *et al*, 2004, 2006; Visser *et al*, 2006; Jia *et al*, 2007; Melamed *et al*, 2008, Semba *et al*, 2009) that had examined the association between serum 25(OH)D concentration and all-cause mortality. Overall, these studies reported that concentrations < 30 nmol/L were associated with an increased mortality risk, which decreased as serum 25(OH)D concentration increased. However, 3 of the studies (Visser *et al*, 2006; Jia *et al*, 2007; Melamed *et al*, 2008) suggested a "U" or "reverse J" shaped dose-response relationship, with a slight increase in all-cause mortality at the highest serum 25(OH)D concentrations. Sambrook *et al* (2004, 2006) found no relationship between serum 25(OH)D concentration and mortality risk and Semba *et al* (2009) did not observe a U-shaped relationship but serum 25(OH)D concentrations in the highest exposure category was 64 nmol/L.
586. A meta-analysis of 14 prospective cohort studies (Zittermann *et al*, 2012⁶⁰) reported a summary RR for mortality of 0.71 (95% C, 0.50-0.91) for highest (≥ 75 nmol/L) vs lowest (< 50 nmol/L) categories of serum 25(OH)D concentration. In the parametric model, the estimated summary RRs (95% CI) for mortality were 0.86 (0.82-0.91), 0.77 (0.70-0.84), and 0.69 (0.60-0.78) for individuals with an increase in serum 25(OH)D concentration of 12.5, 25 and 50 nmol/L respectively, from a median reference category of 27.5 nmol/L. There was no significant decrease in mortality risk when serum 25(OH)D concentrations were 87.5 nmol/L above the reference category.

Pregnancy and lactation

587. Data on adverse effects of vitamin D intakes during pregnancy or lactation are lacking. No adverse effects were observed in 2 studies (Wagner *et al*, 2006; Hollis *et al*, 2011) which supplemented pregnant women with vitamin D doses ≥ 100 μ g/d (4,000 IU).

⁶⁰ Included Visser *et al* (2006); Jia *et al* (2007), Melamed *et al* (2008) and Semba *et al*. (2009).

Infants and children

588. A disorder termed idiopathic infantile hypercalcaemia (IIH) was first recognised in the 1950s when a small number of infants presented with failure to thrive, vomiting, dehydration, fever and nephrocalcinosis (Schlingmann *et al*, 2011). The outbreak was attributed to increased doses of vitamin D (up to 100 µg/4,000 IU per day) from infant formula and fortified milk. Fortification levels of vitamin D in cod liver oil concentrate, dried milk powder, infant cereals and evaporated milk products was subsequently reduced. Vitamin D intakes of infants in the 1960s (6.25-30 µg/d) was found to be substantially lower than in the 1950s (100 µg/day) and incidence of hypercalcaemia in infants had almost halved (Bransby *et al*, 1964). Occasional case reports of infantile hypercalcaemia have been published since, but these have related to specific genetic polymorphisms (see full COT statement).
589. Early studies (Jeans & Stearns, 1938) suggested that excess vitamin D could reduce linear growth in infants but this was not observed at doses up to 54 µg/d (Fomon *et al*, 1966). The absence of effect was supported by a large prospective study (Hyppönen *et al*, 2008) of Finnish children (n=10,060) supplemented with 50 µg/d (2,000 IU) of vitamin D. Growth was also not affected in breast-fed children whose mothers were given 25 or 50 µg/d (1000 or 2000 IU) of vitamin D from birth (Ala-Houhala *et al*, 1986). Calcium concentration in studies where it was measured, was unaffected by vitamin D supplementation.
590. A number of studies in babies and infants have explored the effect of vitamin D supplementation on serum 25(OH)D concentration (Ala-Houhala *et al*, 1986), Vervel *et al*, 1997, Zeghoud *et al*, 1997, Gordon *et al*, 2008). Various regimens of vitamin D supplementation were administered (highest dose was 1250 µg/twice weekly for 6 weeks) but hypercalcaemia was not observed.
591. Fewer data are available for older children but hypercalcaemia was not observed in groups of children (n=8/9; age, 10-17 y) receiving 350 µg (14,000 IU)/week of vitamin D₃ for 8 weeks (Maalouf *et al*, 2008). Similar findings were reported in another study by the same group (El Hajj Fuleihan *et al*, 2006) in which children (n=340) received weekly doses of vitamin D₃ (35 or 350 µg/1400 or 14,000 IU) or placebo for 1 year.

Setting a TUL

592. A TUL (or UL) is the maximum intake that can be consumed every day over a life time without appreciable risk to health. TULs apply to specified population groups but may not be protective of individuals within those groups who have identifiable medical disorders that cause them to be unusually vulnerable to a particular substance. TULs can be established using either human or animal data on adverse effects and incorporate uncertainty factors as appropriate.
593. The COT concluded that best established adverse effect of high vitamin D intakes is hypercalcaemia and that this endpoint should be the critical outcome on which to base TULs for vitamin D. Evidence for other potential adverse effects, which might occur at lower exposures, was considered to be inconsistent.

Adults

594. Based on data from 2 studies relating to hypercalcaemia (Heaney *et al*, 2003; Barger-Lux *et al*, 1998) EFSA set a no-observed-adverse-effect-level (NOAEL) of 250 µg/d (10,000 IU). Applying an uncertainty factor of 2.5 (to account for inter-individual variations in sensitivity and because the NOAEL was derived from 2 small studies), EFSA established a TUL for vitamin D of 100 µg/d (4,000 IU), which is in agreement with the UL for adults set by the IOM in 2011. The COT did not identify any additional studies showing an increased risk of hypercalcaemia at doses lower than the NOAEL of 250 µg/d (10,000) set by EFSA, and agreed that a TUL of 100 µg/d (4,000 IU) was appropriate for adults (18+ y).

595. The TUL does not distinguish between total and supplementary vitamin D intake since dietary intakes make only a small contribution to total exposures at the TUL.

Pregnancy and lactation

596. Neither the IOM nor EFSA adjusted the UL/TUL to take account of pregnancy or lactation. The COT agreed with this position and the TUL of 100 µg/d (4,000 IU) set for adults (18+ y) was considered appropriate for pregnant and lactating women.

Infants and children

597. The ULs set by the IOM for infants aged 0-6 and 6-12 months of 25 and 38 µg/d respectively were based on the studies on growth (Fomon *et al*, 1966; Jeans *et al*, 1938) and considerations about IIH. EFSA (2012) retained the previous TUL of 25 µg/d (1,000 IU) (set in 2003) for children aged 0-12 months, taking particular account of the studies by Fomon *et al* (1966), Jeans *et al* (1938) and Hypönnen *et al* (2011). The COT agreed that the TUL of 25 µg/d (1,000 IU) of vitamin D set by EFSA, for infants aged 0-12 months, was appropriate.

598. The IOM (2011) noted that data were not available for specific age groups other than adults and infants and therefore scaled down the adult UL of 100 µg to 62.5 µg/day for children aged 1-3 y and 75 µg/day for children aged 4-8 y. The ULs set for children and adolescents aged 9-18 years were the same as those for adults. EFSA observed that the studies by Maalouf *et al* (2008) and El-Hajj Fuleihan *et al* (2006) had shown that intakes up to 50 µg/d did not lead to hypercalcaemia and assumed that adolescents (in the phase of rapid bone formation) would not have a lower tolerance for vitamin D than adults. A TUL of 100 µg/d was therefore set for children aged 10-17 y. A TUL of 50 µg/d was agreed for children aged 1-10 y to take account of their smaller body size.

599. COT agreed that the TULs for vitamin D set by EFSA, of 50 and 100 µg/d (2,000 and 4,000 IU) for children aged 1-10 y and 11-17 y respectively, were appropriate.

Groups in which the TULs may not be protective

600. The TULs for vitamin D intake proposed for children and adults in the general UK population might not be protective for individuals with medical disorders that pre-dispose to hypercalcaemia. These include: normocalcaemic hyperparathyroidism; granulomatous diseases such as sarcoidosis and tuberculosis; and genetic pre-disposition such as occurs in IIH.

Single and/or occasional doses of vitamin D

601. Results from most controlled studies in which occasional high doses of vitamin D have been administered suggest that serum 25(OH)D concentrations would not reach levels associated with toxicity. One study of infants (aged 1-20 months), however, reported that vitamin D₂ doses of 15,000 µg (600,000 IU) every 3 months increased serum 25(OH)D concentration by up to 1000 nmol/L and hypercalcaemia occurred in 34% of participants.

602. The COT concluded that vitamin D doses of 7500 µg (300,000 IU) at intervals of 3 months or longer would not be expected to cause adverse effects in adults. There is greater uncertainty about the effects of larger doses, which might cause hypercalcaemia in some individuals, even if given infrequently. The data were insufficient to specify a safe upper limit for single doses in children but the limited available information suggests that toxicity could occur in infants from at doses ≥ 15,000 µg (600,000 IU).

Summary & conclusions

603. Acute and chronic exposure to excess vitamin D intake can result in hypercalcaemia, demineralisation of bone, soft tissue calcification and renal damage. It is the most appropriate endpoint on which to base TULs for vitamin D since adverse effects that might occur at lower doses, through other mechanisms, have not been reliably established.
604. TULs for vitamin D, of 100 µg/d (4,000 IU) for adults and children aged 11-17 y, 50 µg/d (2000 IU) for children aged 1-10 y, and 25 µg/d (1,000 IU) for infants, as recommended by EFSA, are considered appropriate. The TULs do not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D are make only a small contribution to total exposures at the TULs.
605. The TULs proposed may not provide adequate protection for individuals with medical disorders that pre-dispose to hypercalcaemia. These include normocalcaemic hyperparathyroidism, granulomatous diseases (e.g., sarcoidosis and tuberculosis) and genetic pre-disposition (e.g., IIH).
606. Doses of 7500 µg (300,000 IU) at intervals of 3 months or longer would not be expected to cause adverse effects in adults but there is greater uncertainty about the effects of larger doses, which might cause hypercalcaemia in some individuals. There are insufficient data to specify a safe upper limit for single doses in children but the limited information that is available suggests that toxicity could occur in infants at doses $\geq 15,000$ µg (600,000 IU).

8. Dietary vitamin D intakes and serum/plasma 25(OH)D concentrations of the UK population

607. Nationally representative data on vitamin D intakes and serum 25(OH)D concentrations of the general population in the UK were drawn from the National Diet and Nutrition Survey (NDNS) rolling programme, a continuous survey of diet and nutrition in adults and children aged 18 months upwards. The results presented here are based on a UK representative sample of 3450 adults aged 19 years and over and 3378 children aged 1½-18 years collected over years 1-4 combined (2008/09 to 2011/12) (Bates *et al* 2014). Data on institutionalised adults (1994/5) were obtained from the NDNS of people aged 65y and over (Finch *et al*, 1998). Representative data for Scotland were obtained from the NDNS Scotland report 2008/09-2011/12. This report is based on data collected from the boosted sample in Scotland (867 adults and 828 children).
608. Data on low income populations in the UK (aged 2 years and above) in 2003-2005 were obtained from the Low Income Diet and Nutrition Survey (LIDNS) (Nelson *et al*, 2007a; Nelson *et al*, 2007b).
609. Data on infants and young children (aged 4-18 months) were obtained from the 2011 UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC) (Lennox *et al*, 2013).
610. Additional data were obtained from the Health Survey for England (HSE) and the Scottish Health Survey (SHS).
611. Nationally representative data are not available for pregnant women. Data for pregnant women were obtained from UK based cohort studies.

Assessment of vitamin D intakes

612. In the NDNS rolling programme, diet was assessed by a food diary of all foods and drinks consumed over 4 consecutive days. Since dietary surveys are reliant on self-reported measures of intake, misreporting of food consumption (generally under-reporting) is known to be a problem in all dietary surveys, including the NDNS. This is an important consideration in the interpretation of the findings.
613. In the NDNS rolling programme, reported total energy intake in children and adults (4-65+ y) was on average 12-34% lower than reported total energy intake measured by the doubly labelled water (DLW⁶¹) technique. The discrepancy is probably due to a combination of underreporting of actual consumption and changing the diet during recording period. It is not possible to extrapolate these estimates of underreporting of energy intake to individual foods or nutrients nor to correct or adjust intake estimates to take account of under-reporting.
614. Another problem with assessing vitamin D intakes is that it is found in few foods. Therefore, consumption/lack of consumption of vitamin D containing foods during the recording period could have a substantial impact on estimates of habitual intakes. This would be more pronounced for shorter rather than longer recording periods.
615. There is no evidence of under reporting for vitamin D specifically.

Dietary sources of vitamin D

616. In the DNSIYC, infant specific foods were the largest single source of dietary vitamin D, with infant formula the main contributor to vitamin D intakes in non-breast fed infants (aged 4-18 months).
617. In the NDNS, meat and meat products was the major contributor to vitamin D intake for all age groups, except children aged 1.5-3 years, providing 23-35% of intake. Milk and milk products were the major

⁶¹ The DLW technique is included as an objective biomarker to validate energy intakes estimated from reported food consumption.

contributors to vitamin D intake for children aged 1.5-3 years, providing 24%. 'Fat spreads', most of which are fortified with vitamin D, contributed 19-21% to intakes across the age groups. Cereals and cereal products provided 13-20% of intake across the age groups, from fortified breakfast cereals and from 'buns, cakes, pastries and fruit pies' (via fats and eggs used as ingredients).

618. Fish and fish dishes (mainly from oily fish) made a greater contribution to the vitamin D intake of adults (17-23%) compared to children (8-9%).
619. In LIDNS the main sources of vitamin D were meat and meat products for adults (30%) and children (32%) and fat spreads (26%). Cereals and cereal products made a greater contribution to vitamin D intakes of children than older children (about 20% in children aged 2-10 years compared with around 15% in children aged 11-18 years).
620. The vitamin D content of the main dietary sources of vitamin D is provided in Table 1.

TABLE 1- Vitamin D content of dietary sources of vitamin D

Food	Mean vitamin D content (µg/ 100g)
Fish	
Herring (<i>grilled</i>)	16.1
Salmon (<i>farmed, grilled</i>)	7.8
Salmon (<i>farmed, steamed</i>)	9.3
Salmon (<i>pink, canned in brine, drained</i>)	13.6
Salmon (<i>cold & hot smoked</i>)	8.9-11
Mackerel (<i>grilled</i>)	8.5
Mackerel (<i>smoked</i>)	8.2
Sardines (<i>grilled</i>)	5.1
Sardines (<i>canned in brine, drained</i>)	3.3
Tuna (<i>baked</i>)	3.1
Tuna (<i>canned in brine, drained</i>)	1.1
Eggs	
Eggs (<i>whole, boiled</i>)	3.2
Eggs (<i>yolk, boiled</i>)	12.6
Meat	
Beef (<i>rump steak, fried</i>)	0.7
Fortified foods	
Bran flakes	4.6
Cornflakes	4.7
Rice cereal	4.6
Fat spreads (<i>reduced fat 62-75% polyunsaturated</i>)	7.5

Taken from *The Composition of Foods*, 7th edition (Finglas et al, 2015)

Vitamin D intakes in the UK (see tables in Appendix 2)

621. RNIs for vitamin D were only set for specific groups at risk of insufficient sunshine exposure: infants and children aged 0-3 years and adults over 65 years (DH, 1991). Intakes in this section are compared with the current RNIs for these population groups.

Infants and young children

622. Mean intakes of vitamin D from food sources was higher in non breast fed infants compared to breast fed infants (excluding intake from breast milk, as the vitamin D content of breast milk is unknown).
623. For non breast fed infants, mean daily intakes of vitamin D were 9.8 µg (4-6 months), 8.7 µg (7-9 months), 7.5 µg (10-11 months) and 3.5 µg (12-18 months). For breast fed infants (excluding breast milk) mean daily intakes were 3 µg (4-6 months), 3.2 µg (7-9 months), 2.7 µg (10-11 months) and 1.8 µg (12-18 months).

624. Mean intakes for non breast fed infants aged 4-18 months were above the RNI except at 12-18 months, where mean intakes were 55% of the RNI from all sources. For breast fed infants, intakes of vitamin D from all sources (excluding breast milk) were below the RNI at 41% (4-6 months), 52% (7-9 months), 54% (10-11 months) and 37% (12-18 months).

Children

625. For children aged 1.5-3 years, mean daily intake of vitamin D was 1.9 µg from food sources and 2.3 µg from all sources (including supplements). Mean vitamin D intake from all sources was 32% of the RNI for children aged 1.5-3 years.

626. The mean daily vitamin D intake for children aged 4-10 years was 2 µg from dietary sources and 2.7 µg from all sources (including supplements).

627. In the LIDNS, the mean daily vitamin D intake (from food sources only) for boys and girls aged 2-10 years was 2 and 1.74 µg respectively. Mean daily intakes were 22% of the RNI for children aged 2-10 years⁶².

Adolescents

628. In adolescents aged 11-18 years the mean daily vitamin D intake was 2.1 µg from dietary sources and 2.4 µg from all sources (including supplements).

629. In the LIDNS, the mean daily vitamin D intake (from food sources only) for boys and girls aged 11-18 years was 2.4 and 2 µg respectively.

Adults 19-64y

630. For adults aged 19-64 years, mean daily intake of vitamin D from dietary sources was 2.8 µg. Vitamin D supplements increased mean daily intakes to 3.9 µg in men and 3.4 µg in women.

631. In the LIDNS, mean daily vitamin D intakes (from food sources only) for men aged 19-49 and 50-64 years were 3.0 and 3.7 µg respectively; for women aged 19-34, 35-49 and 50-64 years mean daily intakes were 2.2, 2.5, 2.8 µg respectively .

Adults 65y and over

632. The mean daily vitamin D intake from dietary sources in older adults (65 years and over) was 3.3 µg. Dietary supplements containing vitamin D made the largest contribution to intakes in this age group, increasing daily mean intakes to 5.1 µg in men and 5.2 µg in women. Mean intakes for adults aged 65 years and over the mean intake was 51% of the RNI.

633. For institutionalised adults, mean daily vitamin D intake of men was 3.87µg (39% of RNI) from all sources and 3.79 µg (38% of RNI) from food sources; for women, mean daily intake was 3.36 µg (34% of RNI) from all sources and 3.31 µg (33% of RNI) from food sources

634. In the LIDNS, mean daily vitamin D intakes (from food sources only) for adults aged 65+ years were 3.4 µg for men and 2.6 µg for women. Mean daily intake of vitamin D was 34% of the RNI for men and 26% for women.

Serum/plasma 25(OH)D concentrations in the UK (see tables in Appendix 2)

Assessment of serum/plasma 25(OH)D concentration

635. Serum/plasma 25(OH)D concentration reflects the availability of vitamin D in the body from both dietary and endogenous sources (see section x). In the UK a serum/plasma 25(OH)D concentration < 25nmol/L is currently used to indicate low vitamin D concentrations (DH, 1991).

⁶² No RNIs were set for children aged 4-10 years.

636. The main problems associated with the methods used for measuring serum 25(OH)D concentration, include accuracy and variability. Measurements can vary considerably depending on the type of assay used across different concentration ranges. There is also a lack of agreement between different laboratories using the same methods. Measurements of 25(OH)D concentrations from different surveys therefore may not be comparable. (For further details see section x)

637. Blood collection within the NDNS rolling programme and HSE and SHS is spread evenly across the year. In the DNSIYC blood samples were collected between February and August.

Infants

638. Mean plasma 25(OH)D concentration in infants aged 5-11 months was 68.6 nmol/L; 6% had concentrations < 25 nmol/L. For infants aged 12-18 months, mean concentration was 64.3 nmol/L; 2% had concentrations < 25 nmol/L. Out of those infants aged 5-11 months with a plasma 25(OH)D concentration < 25 nmol/L, all were still breastfeeding at the time of the stage 1 interview; none of the infants aged 12-18 months with a 25(OH)D concentration below 25 nmol/L were still breastfeeding.

639. Mean plasma 25(OH)D concentration in boys and girls aged 1.5-3 years was 58.1 nmol/L; 7.5% had concentrations < 25nmol/L, however this is based on a small sample size (n=42).

Children and adolescents

640. Mean plasma 25(OH)D concentration in boys and girls aged 4-10 years was 52.3 nmol/L and 48 nmol/L respectively. 12.3% of boys and 15.6% of girls had a plasma 25(OH)D concentrations < 25 nmol/L.

641. Mean plasma 25(OH)D concentration for boys and girls aged 11-18 years was 44.9 nmol/L and 41.1 nmol/L respectively; out of these, 19.7% of boys and 24.4% of girls had a plasma 25(OH)D concentration < 25 nmol/L.

642. In LIDNS, mean plasma 25(OH)D concentration was 43.5 nmol/L for boys and 39.6 nmol/L girls aged 11-18 years. Eight percent of boys and 23% of girls had a plasma 25(OH)D concentration < 25 nmol/L.

Adults 19-64y

643. In the NDNS, the mean plasma 25(OH)D concentration for adults (19-64 years) was 43.5 nmol/L for men and 47.3 nmol/L for women; 24% of men and 21.7% of women had plasma 25(OH)D concentrations < 25 nmol/L.

644. In the LIDNS, mean plasma 25(OH)D concentration for adults aged 19-64 years was 43-46 nmol/L for men and 34-49 nmol/L for women; 18-25% of men and 14-24% of women had plasma 25(OH)D concentrations < 25 nmol/L.

Adults over 65 years

645. The mean plasma 25(OH)D concentration in adults 65+ years was 47 nmol/L in men 42.5 nmol/L in women; 16.9% of men and 24.1% of women had a plasma 25(OH)D concentration < 25 nmol/L (NDNS 2008/09-2011/12).

646. For institutionalised adults the mean plasma 25(OH)D concentration was 33.7 nmol/L in men and 32.5 nmol/L in women; 38% of men and 37% of women had a concentration < 25 nmol/L).

647. The HSE 2005 breaks down serum 25(OH)D concentration into narrower age bands. For men aged 65-69, 70-74, 75-79, 80-84 and 85+ years, mean serum 25(OH)D concentrations were 53.3, 55.6, 51.6, 48.6 and 48.2 nmol/L respectively. For women aged 65-69, 70-74, 75-79, 80-84 and 85+ years, mean serum 25(OH)D concentrations were 52.4, 51.7, 43.5, 44.8 and 42.3 nmol/L respectively.

648. In the LIDNS, mean plasma 25(OH)D concentration in older adults (65+ years) was 52.8 nmol/L in men and 44.2 nmol/L in women; 14% of men and women had a plasma 25(OH)D concentration < 25 nmol/L.

25(OH)D concentration by season

649. For all age groups mean 25(OH)D concentrations were lowest during the winter months (January-March) and highest in the summer months (July-September). For children (4-10 years) mean plasma 25(OH)D concentration was 37.2 nmol/L in the winter months and 66 nmol/L in the summer. For older children and adolescents (11-18 years) mean plasma 25(OH)D concentration was 31.5 nmol/L in the winter and 52.3 nmol/L in the summer. For adults aged 19-64 years the mean 25(OH)D concentration in the winter was 34.8 nmol/L and 57.5 nmol/L in the summer. For adults aged 65+ years the mean plasma 25(OH)D concentration in winter was 40.5 nmol/L and 50.5 nmol/L in the summer.

650. The proportion with plasma 25(OH)D concentration < 25 nmol/L in the winter months was: children (4-10 years), 31%; older children and adolescents (11-18 years), 40%; adults aged 19-64 years, 39%; and adults aged 65+ years, 29%. The proportion with plasma 25(OH)D concentration < 25 nmol/L in the summer months was: children (4-10 years), 2%; older children and adolescents (11-18 years), 13%; adults aged 19-64 years, 8%; and adults aged 65+ years, 4%.

651. Plasma 25(OH)D concentration by season was also measured in the HSE (2010) and the SHS (2010-2011). For adults (aged 16+ years), concentration was lowest in the winter months compared to the summer months in both England and Scotland; it was also lower in Scotland compared to England in all seasons. In the winter months mean plasma 25(OH)D concentration in England was 33.1 nmol/L and 27.9 nmol/L in Scotland, increasing in the summer months to 60.1 nmol/L in England and 51.3 nmol/L in Scotland. In England, the proportion with plasma 25(OH)D concentration below 25 nmol/L was 42% in winter and 7% in summer; in Scotland, the proportion with plasma 25(OH)D concentration below 25 nmol/L was 54% in winter and 17% in summer.

652. A cohort study of South Asian women (n=86) living in Southern England (Darling *et al*, 2012; 2013) found that 75% and 53% had a plasma 25(OH)D concentration below 25nmol/L in winter and summer respectively. Another cohort study of pregnant women in North West London (n=346) (McAree *et al*, 2013) reported that the proportion with a plasma 25(OH)D concentration below 25nmol/L was 49% in winter and 29% in summer.

Serum 25(OH)D concentration by region

653. The HSE (2010) analysed serum 25(OH)D concentration by region and season. The percentage of people with serum 25(OH)D concentration < 25 nmol/L was lowest in all regions during the summer months (around 5-7%). During the winter months, 46% of people in the Midlands and North, 38% in the South (including London) and 35% in the South (excluding London) had a serum 25(OH)D concentration < 25 nmol/L.

654. Data from NDNS Scotland was not split by season due to small sample sizes. The mean plasma 25(OH)D concentration was 47 nmol/L for children aged 4-10 years, 37 nmol/L for boys and girls aged 11-18 years, 40 nmol/L for adults aged 19-64 years and 42 nmol/L for adults aged 65+ years.

655. The proportion of children who had a 25(OH)D concentration < 25 nmol/L was 9% for children aged 4-10 years, 26% for children aged 11-18 years, 33% of adults aged 19-64 years and 29% of adults aged 65+ years.

656. The percentage of the population with plasma 25(OH)D concentrations < 25 nmol/L was higher in some age groups in Scotland compared with the UK: adults 19-64 years (32.5% in Scotland compared with 22.8%

in the UK), older adults aged 65 years and over (29.4% in Scotland; 21.0% in the UK) and boys aged 11-18 years (29.0% in Scotland; 19.7% in the UK).

Serum 25(OH)D concentration during pregnancy

657. A study in North West London analysed serum 25(OH)D concentrations of pregnant women (n=346; mixed ethnicity) by season (McAree *et al*, 2013). Mean concentrations were 38 nmol/L in summer (July-September), 38 nmol/L in autumn (October-December), 26 nmol/L in winter (January-March) and 32 nmol/L in spring (April-June). The percentage with serum 25(OH)D concentration < 25 nmol/L ranged from 29% in the summer to 49% in the winter.
658. In the Southampton Women's Survey, blood samples of pregnant women (n=977; predominantly white) were taken at 35 weeks gestation. The median serum 25(OH)D concentration was 62 nmol/L and 35% had a serum 25(OH)D concentration < 50 nmol/L (Crozier *et al*, 2012).
659. Blood samples (taken throughout the year) were also available for pregnant women (n=3690; predominantly white) taking part in the ALSPAC⁶³ study 1991/1992 (Lawlor *et al*, 2013). Median serum 25(OH)D concentrations were 55.1, 60.1 and 67.4 nmol/L in the first, second and third trimesters respectively. In the third trimester, 34% of women had a serum 25(OH)D concentration < 50 nmol/L and 6% < 27.5 nmol/L.
660. A study in Aberdeen (Haggarty *et al*, 2012) evaluated plasma 25(OH)D concentrations of pregnant women (n=1205; mean age at delivery, 31.5 ± 5 y; predominantly white) at 19 weeks gestation. Mean concentrations were 53 nmol/L in summer (June-August), 34 nmol/L in autumn (September-November), 34 nmol/L in winter (December-January) and 40 nmol/L in spring (March-May). The percentage with plasma 25(OH)D concentration < 25 nmol/L were 25% in summer, 43% in spring, 60% in autumn and 76% in winter.

Serum 25(OH)D concentration by ethnicity

661. The HSE (2010) analysed serum 25(OH)D concentration by ethnicity (categorised as White, Mixed, Asian, Black and other). Mean serum 25(OH)D concentration was highest in white adults (16 years +): 45.8 nmol/L compared to 20.5 nmol/L for Asian adults and 27.7 nmol/L for black adults. The sample size was too small for mixed and other ethnic groups.
662. The 1996 Asian Infant Feeding Survey (Lawson & Thomas, 1999) measured serum 25(OH)D concentration in Asian children (aged 2 years; n=618) in England. The mean serum 25(OH)D concentration in Bangladeshi infants was 42.1 nmol/L; 20% had concentrations < 25 nmol/L and 13% < 20 nmol/L. The mean serum 25(OH)D concentration in Pakistani infants was 36.2 nmol/L; 34% had concentrations < 25 nmol/L and 18% < 20 nmol/L. The mean 25(OH)D concentration in Indian infants was 42.2 nmol/L; 25% had concentrations < 25nmol/L and 13% had concentrations < 20 nmol/L.
663. The D-Fines⁶⁴ study (Darling *et al*, 2012; 2013) measured serum 25(OH)D concentration in South Asian and white women living in Southern England throughout the year. Overall, the South Asian women had lower serum 25(OH)D concentrations than white women and a higher percentage had serum 25(OH)D concentrations < 25 nmol/L. In autumn 2006 to spring 2007, 80-72.7% of South Asian women had a 25(OH)D concentration < 25 nmol/L compared to 2-10% of white women. The lowest mean 25(OH)D concentration was recorded in the winter months (20.2 nmol/L for South Asian women; 43.7 nmol/L for white women).

⁶³ Avon longitudinal survey of parents and children.

⁶⁴ Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England.

664. In Scotland, Haggarty *et al* (2012) reported that plasma 25(OH)D concentrations in a cohort of pregnant women (n=1205) were significantly lower (-23 nmol/L; p< 0.001) in women from minority ethnic groups (n=42) compared to white women.

Serum 25(OH)D concentration by BMI

665. The HSE (2010) analysed serum 25(OH)D concentrations by BMI (kg/m²). For adults, mean serum 25(OH)D concentration were: BMI < 25, 47.4 nmol/L (33% < 25 nmol/L); BMI of 25-30, 45.7 nmol/L (28% < 25 nmol/L); BMI > 30, 39.8 nmol/L (38% < 25 nmol/L).

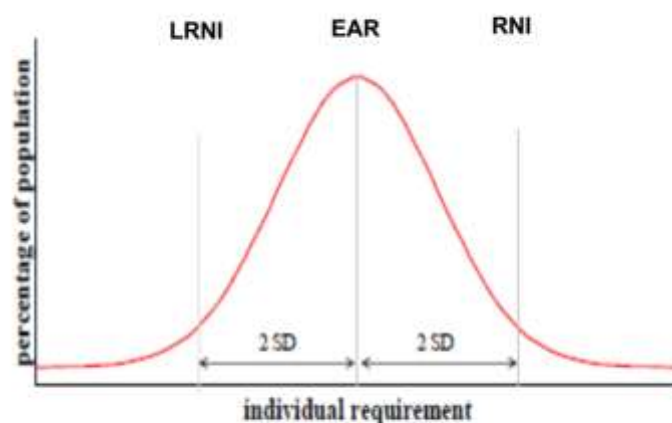
666. Haggarty *et al* (2012) found no association between body weight or BMI on plasma 25(OH)D concentrations during pregnancy.

9. Review of DRVs for vitamin D

Brief overview of DRVs

667. Dietary Reference Values (DRVs) for food energy and nutrients provide benchmark levels of nutrient requirements in the UK (DH, 1991). Although information is usually inadequate to calculate the accurate distribution of requirements for a nutrient in a group of individuals, it has been assumed to be normally distributed (DH, 1991). This gives a notional mean requirement or *Estimated Average Requirement* (EAR); an intake at which about half of a group of people will usually need more than this amount to meet requirements, and half will need less with the inter-individual variability around this (see figure 8). The *Reference Nutrient Intake* (RNI) is a point on the distribution that is two notional standard deviations (SDs) above the EAR, representing an amount that is enough, or more than enough, for approximately 97.5% of people in a group. The *Lower Reference Nutrient Intake* (LRNI), a point on the distribution that is two notional SDs below the EAR, represents the lowest intakes which will meet the needs of some individuals in the group (those who have low needs; approximately 2.5%). Intakes below the LRNI are almost certainly inadequate for most individuals.

FIGURE 8: Dietary reference values – definitions



668. The DRVs were revised by COMA in 1991 (DH, 1991). While the 3 benchmark DRVs (LRNI, EAR and RNI) were used to set requirements for most nutrients, this was not the case for vitamin D. Due to the particular nature of vitamin D, which is obtained from UVB exposure as well as from food and supplements, DRVs were not considered necessary for most of the UK population (e.g., those aged 4-64 years). Instead, the assumption was that vitamin D synthesised dermally in summer through sunlight exposure was sufficient to maintain serum 25(OH)D concentrations above 25 nmol/L⁶⁵ in the following winter months. An RNI only was established for the following population age groups: 0-6 months (8.5 µg/d); 7 months-3 years (7.0 µg/d); and 65+ years (10 µg/d). An RNI was also set for pregnant and lactating women (10 µg/d) and Asian women and children (10 µg/d).

⁶⁵ The current threshold used to define the concentration below which risk of vitamin D deficiency is considered to increase.

Review of current DRVs for vitamin D

669. The purpose of reviewing the current DRVs for vitamin D intake set by COMA (DH, 1991) was to consider whether they are still appropriate to ensure vitamin D adequacy of the UK population in the context of current lifestyles and public health advice (see paragraphs 125-126). As part of this review, the evidence on vitamin D and various health outcomes at different life stages and in different population groups was considered. Evidence from RCTs and prospective cohort studies was preferred in terms of informing the setting of DRVs, however evidence was also considered from other study types (including case control and cross sectional studies) when information from RCTs and cohort studies was lacking.

Selection of health outcome and serum 25(OH)D concentration to use as the basis for setting DRVs

670. Evidence on a range of musculoskeletal and non-musculoskeletal health outcomes was considered (see table 2). Detailed analysis of the evidence considered is provided in Chapter 6 and only the summary conclusions are re-iterated in this section.

TABLE 2. Health outcomes considered in terms of informing DRVs for vitamin D

Musculoskeletal outcomes (relevance to life stage groups)
<ul style="list-style-type: none"> • Rickets (<i>infants and children</i>) • Osteomalacia (<i>all adult age groups</i>) • BMC/BMD (<i>all life stages</i>) • Stress fracture risk (<i>younger adults, mainly athletes and military personnel</i>) • Fracture prevention (<i>adults > 50y</i>) • Fall risk (<i>adults > 50y</i>) • Muscle function and power (<i>adults < and > 50y</i>)
Non-musculoskeletal health outcomes
<ul style="list-style-type: none"> • pregnancy & lactation – non-musculoskeletal health outcomes • cancer • CVD & hypertension • all-cause mortality • autoimmune disease • infectious disease • neuropsychological functioning • oral health • age-related macular degeneration

671. Evidence on vitamin D and musculoskeletal health outcomes suggests beneficial effects of vitamin D in reducing the risk of rickets in infants and children, osteomalacia in adults and risk of falling in adults >50y people and in improving muscle strength and function in all adults. Data on vitamin D and non-musculoskeletal health outcomes were considered insufficient at this time to inform the setting of DRVs for vitamin D. Musculoskeletal health was therefore selected as the basis for setting the DRVs for vitamin D.

672. As outlined in Chapter 6, the available data are insufficient to establish a distribution of serum 25(OH)D concentrations for musculoskeletal health, which underpins the establishment of the three DRV estimates (LRNI, EAR, RNI). However, the risk of poor musculoskeletal health appears to increase at serum 25(OH)D concentrations < 25 nmol/L (based on rickets in children, osteomalacia in adults, falls in adults > 50 years and muscle strength and function in adolescents and adults). A serum 25(OH)D concentration of 25 nmol/L was, therefore, selected as the basis for establishing the RNI for vitamin D; i.e., the mean vitamin D intake required to achieve a serum 25(OH)D concentration ≥ 25 nmol/L, throughout the year, by the majority (97.5%) of the population. This threshold was extended to those life-stages where there was

insufficient evidence to identify the serum 25(OH)D concentration associated with risk of poor musculoskeletal health (pregnancy and lactation).

673. A serum 25(OH)D concentration of 25 nmol/L indicates the concentration below which the risk of poor musculoskeletal health is increased and above which the risk is decreased at a population level. It therefore represents a '*population protective*' concentration.
674. Data from the rolling NDNS show that the majority of the UK population achieve a serum 25(OH)D concentration > 25 nmol/L during the summer and this is probably due to skin synthesis of vitamin D in response to sun exposure. However, sun exposure is notably reduced as a contributor to serum 25(OH)D concentrations of the UK population in the winter months. Data from the NDNS and other studies suggest that between 29 and 54% of various population groups in the UK have a serum 25(OH)D concentration < 25 nmol/L in the winter (see paragraph x, chapter 8). In addition, although most people would be expected to synthesise vitamin D in the summer and naturally achieve serum 25(OH)D concentrations \geq 25 nmol/L due to sun exposure, there is a proportion (6-53%) of some population groups in the UK with serum 25(OH)D concentration < 25 nmol/L in the summer (see paragraphs 649-652). Since it is not possible to identify these individuals it is proposed that the RNI should apply throughout the year, otherwise the protection of 97.5% of the population against risk of poor musculoskeletal health will not be achieved.
675. Current recommendations (DH, 1991) assume that enough vitamin D is synthesised dermally in summer through sunlight exposure to maintain serum 25(OH)D concentration above 25 nmol/L in the following winter months (which is why an RNI was not set for individuals aged 4-64 years). However, summer synthesis of vitamin D, facilitating maintenance of winter serum 25(OH)D concentration \geq 25 nmol/L is clearly not occurring for many in the UK population.

Comparison of selected health outcomes and serum 25(OH)D concentration to be used as basis for setting DRVs with those selected by IOM

676. The IOM reviewed the data on vitamin D and a range of health outcomes to assess their suitability as a basis for developing Dietary Reference Intakes (DRIs) for vitamin D. The DRIs (like the DRVs) relate to a distribution of requirements and comprise: an average requirement (estimated average requirement or EAR); the intake likely to meet the needs of about 97.5% of the population (recommended dietary allowance or RDA⁶⁶); the intake above which the potential for harm increases (tolerable upper intake level or UL); and an adequate intake (AI) which is set when available data are too limited to establish an EAR.
677. Bone health was selected by the IOM as the basis for setting an EAR and RDA for vitamin D for all life stage groups, except infants where an AI was specified. Evidence for other health outcomes⁶⁷ was not considered sufficient for informing the DRIs. Conclusions in relation to serum 25(OH)D concentrations and bone health were that the data, overall, suggested that a serum 25(OH)D concentration < 30 nmol/L was associated with: increased risk of rickets, impaired fractional calcium absorption and decreased BMC in children and adolescents, impaired fetal skeletal outcomes, impaired fractional calcium absorption and an increased risk of osteomalacia in young and middle-aged adults and impaired fractional calcium absorption and fracture risk in older adults. A serum concentration of 30 nmol/L was considered to be consistent with the lower end of requirements. It was also concluded that there was a trend for maximal calcium absorption at serum concentrations of 50 nmol/L and little causal evidence for additional benefits on BMD, fracture risk or osteomalacia risk at serum 25(OH)D concentrations > 50 nmol/L.

⁶⁶ The RDA is equivalent to the reference nutrient intake in the UK; i.e., the amount likely to meet the needs of nearly all (97.5%) of the general healthy population, therefore exceeding the requirements of most of the population.

⁶⁷ Cancer, CVD, hypertension, diabetes and metabolic syndrome, falls and physical performance, immune function and autoimmune disorders, infections, neuropsychological functioning and pre-eclampsia.

678. Using this range of 30-50 nmol/L to capture the distribution of vitamin D requirements for bone health, a serum 25(OH)D concentration of 40 nmol/L was selected to be consistent with the median dietary requirement and this concentration was used to establish the EAR intake value for vitamin D as it covered half the population's needs. By convention, adding two SDs to the average requirement would cover the needs of 97.5% of the population (therefore using 40 nmol/L as the average requirement, 50 nmol/L would cover the needs of most individuals in terms of vitamin D and this was used to establish the RDA intake value for vitamin D).
679. The considerations of the current review agree with the IOM in terms of selecting rickets and osteomalacia as the basis for developing DRVs. However, evidence on BMC and fracture risk was considered insufficient to inform the setting of DRVs and calcium absorption was not considered as a health outcome but as an intermediate factor affecting bone health. Evidence on falls and muscle strength and function (which were not used by the IOM in the development of DRIs) has strengthened since publication of the IOM report and these additional outcomes are used here to inform the setting of the DRVs for vitamin D.
680. In the current review, the data were not considered sufficient to establish a distribution of serum 25(OH)D concentrations that would be required to estimate the distribution of requirements for vitamin D intake (i.e., LRNI, EAR, RNI). The threshold serum 25(OH)D concentration of 25 nmol/L was therefore selected as indicative of the serum 25(OH)D concentration below which risk of musculoskeletal health appears to be increased. Since it represents the concentration that the majority of the population (about 97.5%) should be above in terms of protecting musculoskeletal health, it corresponds to an RNI-type value. This approach differs from the serum 25(OH)D concentration of 50 nmol/L selected by the IOM as being consistent with an RDA-type value (i.e., covering the needs of 97.5% of the population).

Modelling exercise

681. Two modelling options for attaining year-round serum 25(OH)D concentration \geq 25 nmol/L were investigated:
- the summer sunshine exposure required to maintain serum concentration \geq 25 nmol/L during winter (i.e., current DH, 1991 approach);
 - the intake of vitamin D, assuming minimal sun availability throughout the year (as per winter), required to maintain serum 25(OH)D concentration \geq 25 nmol/L.

Modelling the summer sunshine exposure required to maintain a winter serum 25(OH)D concentration \geq 25 nmol/L

682. A number of factors affect cutaneous vitamin D synthesis including skin colour, exposed skin area, length and frequency of exposure and latitude (Webb, 2006). The foremost factor affecting skin vitamin D synthesis is availability of UVB radiation (Webb & Engelsen, 2006). In the UK, summer sunlight contains enough UVB for vitamin D synthesis; however, the small amount of UVB in winter sunlight means that vitamin D synthesis is negligible from at least October through March (Webb *et al*, 1988) and serum 25(OH)D concentrations decline throughout the winter.
683. A UK study⁶⁸ (Kazantzidis *et al*, in press) has modelled the duration and intensity of sunlight exposure that would be required by adults in the summer to maintain serum 25(OH)D concentrations \geq 25 nmol/L in the winter.
684. In the first part of the modelling exercise, UVB availability across the UK was calculated using a radiative transfer model that took account of altitude, surface reflectivity, ozone, aerosols and cloud. Satellite data

⁶⁸ This study was specifically commissioned by the Department of Health to inform SACN's review of the DRVs for vitamin D.

(2003-2012) were used to calculate UV wavelengths arriving at the Earth's surface which were then weighted with the different action spectra⁶⁹ (for pre-vitamin D synthesis in the skin, erythema and DNA damage). The model was validated against ground-based spectral solar UV measurements from monitoring sites in Manchester and Reading (biological weightings) and broadband (erythema weighted) UV data from UK-wide sites.

685. Data from previous observation and intervention studies (Rhodes *et al*, 2010; Webb *et al*, 2010; Farrar *et al*, 2013; Kift *et al*, 2013) of healthy adults (20-60 y) with skin types I-IV (white) and V (South Asian) were used to provide data on the response of serum 25(OH)D concentration to UV radiation within the modelling. These data showed that the South Asian cohort needed approximately 2.5 times more UV radiation to produce the same change in serum 25(OH)D concentration as the white-skinned cohort.
686. The shortest and safest exposure regime was selected for use in the modelling. This was based on the fact that the ratio of UVB to UVA is greatest when the sun is high in the sky. Since UVA contributes to erythema risk but not to vitamin D synthesis, the maximum benefit to risk ratio (or vitamin D synthesis to sunburn ratio) is at solar noon. Exposure was therefore restricted to the hours around solar noon. Since solar intensity is also greatest at this time, this reduces the required exposure time. The safe limit of UV for daily noon-time solar exposure was taken as 1 SED for skin types I-IV and 2.75 SED for the skin type V.
687. Using linear regression techniques, it was estimated that 95% of the white-skinned adult population in the UK would maintain a serum 25(OH)D concentration above 25 nmol/L in winter if all individuals achieved a serum 25(OH)D concentration \geq 80.5 nmol/L in September. Year-round serum 25(OH)D concentrations were too low in the South Asian cohort to make a separate recommendation for this group. Even with this extra limitation, modelling showed that the end of summer (August) target for this group was 85.8 nmol/L. This higher target compared to that in white skin types relates to the fact that vitamin D synthesis was assumed to start a little later and end a little earlier in the year because the pigment in the skin reduces UVB radiation reaching 7-DHC, exacerbating the effect of large solar zenith angles and leading to more inefficient production of vitamin D.
688. An estimate of sun exposure area and duration to achieve the \geq 80.5 nmol/L was also performed within the modelling. It was estimated that with 35% skin area exposed (equivalent to wearing modest shorts/skirt and T-shirt) at around noon (12:00-13:00) from March to September, the daily exposure time to reach the end of summer (September) target serum 25(OH)D concentration would be 9 minutes for skin types I-IV (white) and 25 minutes for skin type V (south Asian ethnicity). These exposure durations would not be expected to exceed the sunburn thresholds for skin types I-V.
689. It was further estimated that if the skin area exposed was modified (35% in June-August and 10% in March-May and September [equivalent to hands and face being exposed]), the target serum 25(OH)D concentrations would be met across the country in a typical year for weather but the end of summer serum 25(OH)D concentration would be marginal in Scotland. This means that a summer with particularly poor weather or non-adherence to the exposure regime would reduce the end of summer serum 25(OH)D concentration to below the target concentrations. In addition, if only hands and face (10% surface area) were exposed throughout the year then the target 25(OH)D concentration would not be met.
690. To take account of lower solar radiation at more northerly latitudes, estimated exposure times required to reach end of summer target serum 25(OH)D concentrations (assuming 35% skin area exposure from June-August) ranged from 9-14 minutes for skin type I-IV and 25-38 minutes for skin type V. These estimates would be much higher if exposure was limited to 10% i.e., hands and face.
691. There are important limitations in these estimations. The two main inputs for the model, weather and

⁶⁹ Relative efficacy of different wavelengths in causing an effect.

human behaviour, have numerous combinations. The calculations represent a typical outcome for a normal weather year and for an average person following the specified exposure pattern. The model also assumes exposure in an open environment; however, exposure in urban environments or shade seeking behaviour will reduce the UVB dose received and therefore the likelihood of reaching the target serum 25(OH)D concentration. It also assumes daily exposure regardless of weather. While the modelling takes account of cloud, it does not take account of rainy days. When cloud is thick enough to produce persistent rain the incident UV radiation is significantly reduced. Another assumption is that skin on all parts of the body synthesises vitamin D at the same rate. A further limitation is that the *mean* serum 25(OH)D concentration in winter was used within the modelling to estimate the required increase in serum 25(OH)D concentration in the summer. This means that the estimated sunshine exposure required in the summer to maintain serum 25(OH)D concentration ≥ 25 nmol/L in the winter applies to the average person but would not cover the requirements of 97.5% of the population.

Modelling the vitamin D intakes required to achieve a protective serum 25(OH)D target concentration ≥ 25 nmol/L

692. As outlined in the previous section, using a recommended sunlight exposure as a means of achieving and maintaining serum 25(OH)D concentration ≥ 25 nmol/L for most people is problematic. Taking account of sunlight exposure in setting the RNI (i.e. dietary intake) for vitamin D is complicated by the number of factors that impact on dermal production of vitamin D.
693. The process of translating the target serum 25(OH)D concentration of ≥ 25 nmol/L into an RNI was therefore based on studies that were carried out during winter, in the absence of (or with minimal) UVB radiation of sufficient strength to allow for production of vitamin D in the skin. The RNI, therefore, represents the intake needed to achieve a serum 25(OH)D concentration ≥ 25 nmol/L by 97.5% of the population when UVB exposure is minimal. However, because there are substantial proportions (6-53%) of some population groups in the UK with a serum 25(OH)D concentration < 25 nmol/L in the summer, the RNI is required throughout the year to protect 97.5% of the population, as per DRV convention.
694. Two possible approaches were considered to define the relationship between vitamin D intake and serum 25(OH)D concentration during winter in various age-group(s) in order to inform RNI for vitamin D:
- a. meta-regression approach; and
 - b. approach using data from individual vitamin D RCTs.

(a) Meta-regression approach

695. This approach was used by the IOM to specify DRIs for vitamin D in 2011 (IOM, 2011) and by the Nordic Council of Ministers [NORDEN] to establish Recommended Intakes for vitamin D for the Nordic countries in 2013 (NORDEN, 2013).
696. The advantage of the meta-regression approach is that data from a number of RCTs are used, which avoids over reliance on data from one particular RCT. Group mean or median serum 25(OH)D data from the various intervention arms from each selected RCT are used, together with an estimate of total vitamin D intake (food plus supplemental vitamin D). The disadvantage is that data are combined from RCTs that used a variety of analytical methods to measure serum 25(OH)D concentrations and the impact of different methods on serum 25(OH)D results has been widely reported (Carter, 2011, 2012). Additionally, since group *mean or median* serum 25(OH)D concentrations are used in the meta-regression, the resulting regression line and its 95% confidence intervals provide an estimate of the EAR rather than the RNI. They also do not provide estimates of inter-individual variability in the intakes of vitamin D required to achieve a specific serum 25(OH)D concentration, which would be needed to estimate the intake required to allow 97.5% of individuals to achieve the serum 25(OH)D concentration.

697. Another important limitation with the meta-regression model used by the IOM or Norden, especially in the context of the current DRV exercise, is the lack of representative data at the lower end of the serum 25(OH)D concentration range since there were no RCTs with intervention arms that achieved mean/median serum 25(OH)D concentrations < 25 or even < 30 nmol/L. This means that there are insufficient data from RCTs to estimate directly the vitamin D intake required to achieve a threshold serum 25(OH)D of 25 nmol/L and such data would have to be extrapolated. This was not a problem for either the IOM or NORDEN because both committees selected the higher threshold serum 25(OH)D concentration of 50 nmol/L to use as the basis for the RDA and Recommended Intake values for vitamin D respectively.

(b) Approach using data from individual RCTs

698. The lack of well-characterised inter-individual variability estimates inherent in the meta-regression approach (due to the use of mean/median group responses in the analysis) can be overcome when data from individual vitamin D RCTs and their individual participant-level data are used. With this method it is possible to estimate with more confidence the distribution of intakes required to achieve specified serum 25(OH)D concentrations.

699. This approach was therefore used to determine the RNI for vitamin D using data on the individual response of serum 25(OH)D concentration in winter to increased vitamin D intake from three RCTs in: adults aged 20-40 y; adults aged 64+ y; and adolescent girls aged 11 y (Cashman *et al*, 2008; 2009; 2011). These three RCTs were used because they were conducted in winter at Northern latitudes appropriate to the UK and were specifically designed to characterise the distribution of dietary requirements for vitamin D required for the maintenance of serum 25(OH)D concentrations ranging from 25 to 80 nmol/L, during winter.

700. Data from two of these RCTs⁷⁰ (Cashman *et al*, 2008; 2009) were from adults in the south of the Republic of Ireland (latitude 51°N) and Northern Ireland (latitude 55°N) (NDNS shows that mean serum 25(OH)D concentrations in older adults were ~10 nmol/L lower in the Northern region of UK [55-57°N] than in London and the South East [51°N]). The RCT in girls (Cashman *et al*, 2011) was conducted at 55°N and 60°N (Copenhagen, Denmark, and Helsinki, Finland, respectively) representing latitudes in the UK from Edinburgh to the Shetlands. The total vitamin D intake range in the RCT study designs was specifically selected to provide a range of intakes of vitamin D within the current 2.5th and 97.5th percentiles of intakes for UK adults/older adults (NDNS). The vitamin D intakes of the girls are comparable to those for girls in the UK. The same RCT data were used by the German Nutrition Society in setting the DACH⁷¹ country recommendations for vitamin D but using 50 nmol/L as their basis (German Nutrition Society, 2012).

Further considerations in setting DRVs for vitamin D using the RCTs data

701. The RNI is notionally an intake of a nutrient that is two SDs above the EAR (see paragraph 667) and is therefore derived from a known EAR and the variance around the distribution. In relation to vitamin D, especially the vitamin D intake-serum 25(OH)D concentration relationship, there are sufficient data to estimate directly the vitamin D intake required to maintain serum 25(OH)D concentration above a selected concentration (i.e., 25 nmol/L) over winter at the 97.5th percentile (i.e., the RNI). This direct estimation avoids the requirement to add two SDs to the EAR, which is an approximation⁷².

702. Using data from individual RCTs for adults 20-40 y and adults 64+ y, older people and teenage girls (Cashman *et al*, 2008; 2009; 2011a), it is estimated that a vitamin D intake of 8.3-8.7 µg/d would be

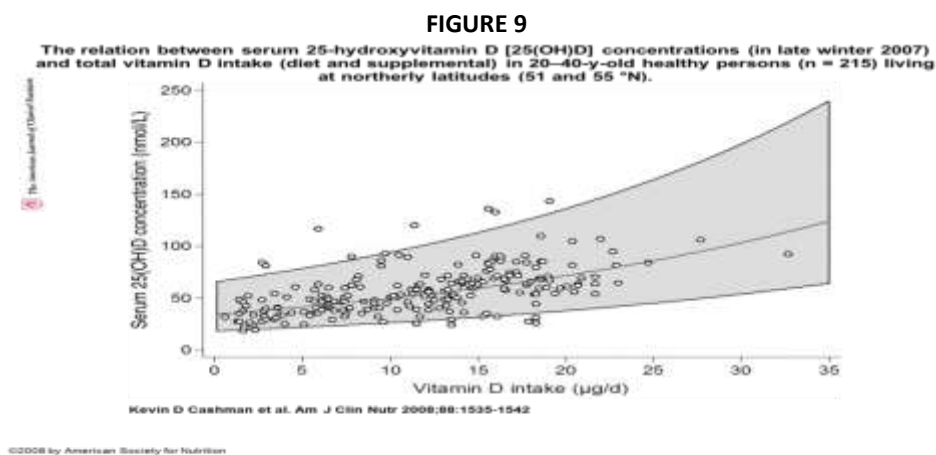
⁷⁰ These RCTs were specifically commissioned and funded by the Food Standards Agency to inform considerations on whether the DRVs for vitamin D (DH, 1991) required re-evaluation.

⁷¹ [D-A-CH arises from the initial letters of the common country identification for the countries Germany (D), Austria (A) and Switzerland (CH)].

⁷² While the EAR is a constituent value within the DRV, the EAR does not exist at the threshold serum 25(OH)D concentration of 25 nmol/L for adults 20-40 y and adults 64+ y (see Tables 3 and 4).

needed to maintain winter serum 25(OH)D concentrations > 25 nmol/L in these individuals (see tables 3-5). While the 97.5th percentile represents the RNI, data on lower percentiles (50th [EAR], 90th and 95th) are also presented.

703. Full details of the regression and mathematical modelling used to derive these intake estimates are provided in the three publications (Cashman *et al*, 2008; 2009; 2011a) but, in brief, the aim of the modelling was to describe the conditional distribution of serum 25(OH)D concentrations at specific values of vitamin D intake in each of the three population subgroups separately. A regression model was used to estimate the variation in serum 25(OH)D concentrations about the mean and Q-Q plots were used to examine the assumption that variation about the predicted value was normally distributed. The distribution for serum 25(OH)D concentration as a function of total vitamin D intake was obtained for each of the three population subgroups separately (see Figure 9 below, as an example). Finally, the dietary requirements for vitamin D to maintain selected percentages of the population above specific serum 25(OH)D concentrations was estimated. In all three age-groups, results were verified using robust regression models that minimised the effect of outliers and heteroscedasticity.



704. The estimated intakes are derived from serum 25(OH)D concentration data measured by immunoassay for adults (20-40 and 64+ y) and by HPLC for adolescent girls (11 y). A comparison of serum 25(OH)D concentrations measured by immunoassay and standardized LC-MS/MS⁷³ has reported that the immunoassay-based concentrations are positively biased (Cashman *et al*, 2013). The two RCTs which used an immunoassay to analyse serum 25(OH)D concentrations (Cashman *et al*, 2008; 2009) suggest that a vitamin D intake of about 9 µg/d is required to achieve a winter serum 25(OH)D concentration ≥ 25 nmol/L (see Tables 3-5). However, reanalysis of serum 25(OH)D concentration from these two RCTs by standardized LC-MS/MS and running the models with these new data suggests that about 12 µg/d is needed to achieve and maintain winter serum 25(OH)D concentration ≥ 25 nmol/L in 97.5% of adults.

705. Although LC-MS/MS is now the preferred method for analysis of serum 25(OH)D concentration (Wallace *et al*, 2010), immunoassays were used in the majority of studies on vitamin D and various health outcomes (including those suggesting risk of musculoskeletal health outcomes is increased at serum 25(OH)D concentrations < 25 nmol/L). Since estimates of the vitamin D intake required to maintain serum 25(OH)D concentration ≥ 25 nmol/L in winter by 97.5% of the population differ according to whether immunoassay or LC-MS/MS analysis (~9 and 12 µg/d respectively) is used, an RNI of 10 µg/d was set between these two estimates.

⁷³ The LC-MS/MS method used in these analysis is certified by the Centers for Disease Control and Prevention's Vitamin D Standardization Certification Program.

RNIs for vitamin D by life-stage

UK general population aged 11 to 65+ years

706. An RNI of 10 µg/d is proposed for the UK general population aged 11-65+ years. The RNI assumes minimal sunshine exposure because the studies used to derive this figure were carried out in winter. This is the amount needed to achieve a serum 25(OH)D concentration \geq 25 nmol/L during winter in 97.5% of the population.
707. Although most people would be expected to synthesise vitamin D and naturally achieve a serum 25(OH)D concentration \geq 25 nmol/L during the summer due to sun exposure, data from the NDNS and other studies suggest that substantial proportions (6-53%) of some population groups in the UK have a serum 25(OH)D concentration $<$ 25 nmol/L in the summer. Since it is not possible to identify these individuals, it is proposed that the RNI should apply throughout the year. This is a precautionary approach to protect population groups and individuals with sustained serum 25(OH)D concentration $<$ 25 nmol/L and to take account of variable exposure to sunshine and diet. It ensures coverage of 97.5% of the population throughout the year.
708. The data used to estimate the vitamin D intake required to achieve a serum 25(OH)D concentration \geq 25 nmol/L were drawn from individual RCTs in adults aged 20-40 y, adults aged 64+ y and adolescent girls (aged 11 y). Dose-response data were not available to allow direct determination of the vitamin D intake needed to achieve a serum 25(OH)D concentration \geq 25 nmol/L in infants and children aged 0-10 years or during pregnancy and lactation. However, the IOM did not find an age-dependent effect of vitamin D supplementation dose (but noted that this finding was based on a limited amount of data). A subsequent Danish RCT of children and adults (n=782), on the effects of vitamin D fortified milk and bread on serum 25(OH)D concentrations (Madsen *et al.*, 2013), reported no difference in treatment effects by age group (4-10, 11-17, 18-40 and 41-60 years). This suggests that data from the RCTs in adults (20-40 years and 64+ years) and adolescent girls (aged 11 years) can be extrapolated to younger age groups.

Pregnancy and lactation

709. Since data are not available to suggest the requirement for an additional increment during pregnancy and lactation, the RNI proposed for the general UK population (10 µg/d) is applicable to pregnant and lactating women.

Infants and children aged 0 to < 4 years

710. Current RNIs for infants and children are 8.5 µg/d for 0-6 months and 7 µg/d for 7 months-3 years (DH, 1991). The RNI for 0-6 months (8.5 µg/d) is based on the amount of vitamin D in infant formula and the RNI for 7 months-3 years (7 µg/d) is based on recommendations of a previous COMA report (DH, 1988⁷⁴) that infants and young children up to 2 years of age should receive supplementary vitamin D, then providing 7 µg/d. It is uncertain how these figures were derived and the paucity of data does not support different supplementation doses for 0-6 months and 7 months-3 years.
711. Since data are not available to relate serum 25(OH)D concentration in the infant clearly to current or long term health, *Safe Intakes*⁷⁵ rather than RNIs are recommended for ages 0-3 years. COMA (DH, 1991) set a Safe Intake for some nutrients if there were insufficient reliable data to set DRVs. Safe Intakes are based on a precautionary approach and reflect the insecurities of the data. They are set on grounds of prudence and are '*judged to be a level or range of intake at which there is no risk of deficiency, and below a level of*

⁷⁴ Present day practice in infant feeding; third report.

⁷⁵ Not to be confused with 'Safe Upper Level' which represents an intake that can be consumed daily over a lifetime without significant risk to health (EVM, 2003) or the 'Tolerable Upper Level': the highest average daily intake of a nutrient intake that is likely to pose no risk of adverse health effects for nearly all persons in the general population (IOM).

where there is a risk of undesirable effects' (DH, 1991).

712. Evidence from the *DNSIYC* shows that out of those infants aged 5-11 months with plasma 25(OH)D concentrations < 25 nmol/L (6%), none were receiving infant formula. Therefore, in pragmatic terms, a Safe Intake range of 8.5-10 µg/d for children aged 0-11 months would accommodate current practice determined by concentrations of vitamin D in infant formula. A Safe Intake of 10 µg/d, based on the RNI for the rest of the UK population, is proposed for children aged 1 to < 4 years. The proposed safe intakes are not additional to vitamin D intakes from infant formula but include the amount contained in formula milk.
713. There is currently no vitamin D RNI for exclusively breast fed infants because it was previously assumed that maternal vitamin D supplementation during pregnancy would provide the infant with adequate vitamin D for the period of exclusive breast feeding, which, together with intake from breast milk, would sustain serum 25(OH)D concentration during the period of exclusive breast feeding. There is limited evidence on exclusively breast fed infants but the available data suggest it is unlikely that an exclusively breast fed infant in the UK would maintain serum 25(OH)D concentrations ≥ 25 nmol/L for 6 months. Therefore the Safe Intakes proposed for non-breast fed infants are also proposed for exclusively breast fed infants (from birth).

Infants and children aged 4 to < 11 years

714. For infants and children aged 4 to < 11 years, there is no evidence to suggest a different value to the RNI set for the rest of the UK population (10 µg/d). An RNI of 10 µg/d is therefore considered appropriate for this age group.

'At risk' groups

715. Individuals in population groups 'at risk' of having a serum 25(OH)D concentration < 25 nmol/L are frail older people, those from ethnic groups with darker skin, those not spending substantial time outdoors and those wearing concealing clothing.
716. There is no evidence to support an additional increment to the RNI for adults aged 65+ years. A subset within this age group, who are at risk of having serum 25(OH)D concentrations < 25 nmol/L, are those more likely to be spending less time outdoors either because of frailty or being institutionalised. However, an additional increment is not considered necessary for population groups who spend less time outdoors or wear concealing clothing because the proposal that the RNI is applicable throughout the year is to take account of those with minimal sunshine exposure who would be at risk of having a serum 25(OH)D concentration < 25 nmol/L in summer.
717. Lower serum 25(OH)D concentrations have been observed in ethnic groups with darker skin. However, darker skin is only one of many factors, including cultural (e.g., wearing concealing clothing) and biological (e.g., genetic background), that might affect serum 25(OH)D concentration. Findings from dose-response RCTs of African Americans in the USA have produced conflicting estimates of the RDA for vitamin D even within a life-stage subgroup and also conflicting data as to whether the RDA estimates differ among African American and white adults (Gallagher *et al*, 2013; Gallagher *et al* 2014; Ng *et al*, 2104) (see paragraphs 169-171). It is also not known if the lower serum 25(OH)D concentrations observed in people with darker skin are associated with any adverse health outcomes since the evidence on health outcomes has been obtained from studies that include predominantly white skinned people. It is uncertain, therefore, whether the vitamin D requirement for people from ethnic groups with darker skin is higher than the RNI of 10 µg/d proposed for the general population. On the basis that there are currently insufficient data to set a higher RNI for people from different ethnic groups, the proposed RNI of 10 µg/d is considered to cover the needs of ethnic groups within the UK population.

718. Evidence suggests that obese people are also at risk of low serum 25(OH)D concentrations. However, there are currently insufficient data to make a different recommendation from that for the general population.
719. If the proposed RNI is achieved by almost all of the UK population, the current distributions of vitamin D intake and serum 25(OH)D concentrations would shift to the right with an increase in mean/median intakes and serum 25(OH)D concentrations. However, it is unlikely that this would lead to those with intakes at the top end of the distribution reaching vitamin D intakes or serum 25(OH)D concentrations that might pose a risk of adverse effects. Findings from a study (Allen *et al*, 2015) which modelled the effect of vitamin D intakes resulting from different fortification scenarios on population groups at risk of vitamin D deficiency⁷⁶, using NDNS (2008-2010) data (n=2127) estimated that a vitamin D intake of 10 µg/d (400 IU) would reduce the proportion of at-risk groups estimated to have intakes below the current RNI from 93% to 50% with no individual exceeding the UL⁷⁷ (EFSA, 2002).

Summary & conclusions

720. The RNI for vitamin D proposed for the UK population is based on protection of musculoskeletal health.
721. A threshold serum 25(OH)D concentration of 25 nmol/L was used as the criterion for establishing the RNI for vitamin D. This concentration represents a '*population protective*' level; i.e., the concentration below which risk of poor musculoskeletal health is increased and above which the risk is decreased at a population level. The RNI was developed to ensure that the majority (97.5%) of the population has a serum 25(OH)D concentration > 25 nmol/L all year round.
722. Sunlight UVB exposure could not be taken into account in setting the RNI because it is not possible to quantify the contribution it made to serum 25(OH)D concentrations within the general population.
723. The process of translating the target serum 25(OH)D concentration of ≥ 25 nmol/L into an RNI for vitamin D was based on RCTs carried out during winter when UVB radiation is absent or minimal.
724. An RNI of 10 µg/d of vitamin D, applicable throughout the year, is proposed for the UK population aged 4 years and above. The RNI assumes minimal sunshine exposure.
725. The RNI of 10 µg/d also includes *at risk* population groups (frail older adults, individuals wearing concealing clothing; those not spending substantial time outdoors; people from ethnic groups with darker skin).
726. A Safe Intake range of 8.5-10 µg/d is proposed for infants aged 0-11 months, including those who are exclusively breast-fed (from birth). The proposed Safe Intake for exclusively breast-fed infants is a change to previous advice.
727. A Safe Intake of 10 µg/d is proposed for children aged 1 to < 4 years.

⁷⁶ For the purposes of this study, at risk groups were defined as young children (aged 18m-3y), women of child-bearing age (aged 15-49y representing pregnant & breastfeeding women) and adults aged ≥ 65 y.

⁷⁷ The ULs used in the analysis by Allen *et al* (2015) were based on the levels set by EFSA in 2002: 0-10y, 25 µg/d; 11-17y & adults, 50 µg/d. The ULs were revised by EFSA in 2012: < 1y, 25 µg/d; 1-10y, 50µg/d; 11-17y & adults, 100 µg/d. The revised ULs were considered appropriate by the COT and accepted as the TULs for the UK (COT, 2014).

TABLE 3: Estimated dietary requirements for vitamin D at selected percentiles in men and women aged 20-40 years (n=215) to maintain serum 25(OH)D above selected concentrations during winter

Serum concentrations > 25 nmol/L and 97.5th percentile are emphasised (in bold) in all tables because of their relevance to current DRV review (i.e. RNI).

Serum 25(OH)D concentration (nmol/L)	50 th percentile ²	90 th percentile	95 th percentile	97.5 th percentile
			<u>µg/d</u>	
>25	-	2.7	5.9	8.7
>30	-	7.6	10.9	13.7
>40	4.1	15.5	18.8	21.7
>50	10.2	21.7	25.0	28.0

¹ Results based on a log-linear model of serum 25(OH)D as a function of vitamin D intake

² The vitamin D intake that will maintain serum 25(OH)D concentrations in 50% of adults aged 20-40 y above the indicated cut-off level during winter.

TABLE 4: Estimated dietary requirements for vitamin D at selected percentiles in free-living men and women aged 64+ years (n=225) to maintain serum 25(OH)D above selected concentrations during winter¹

Serum 25(OH)D concentration (nmol/L)	50 th percentile ²	90 th percentile	95 th percentile	97.5 th percentile
			<u>µg/d</u>	
>25	-	2.6	5.8	8.6
>30	-	6.2	9.4	12.2
>40	1.3	12.7	16.0	18.8
>50	7.1	18.4	21.8	24.7

¹ Results based on a square-root-linear model of serum 25(OH)D as a function of vitamin D intake

² The vitamin D intake that will maintain serum 25(OH)D concentrations in 50% of adults aged 64+ y above the indicated cut-off level during winter.

TABLE 5: Estimated dietary requirements for vitamin D at selected percentiles in adolescent girls (n=144; mean age 11.3 years) to maintain serum 25(OH)D above selected concentrations during winter¹

Serum 25(OH)D concentration (nmol/L)	50 th percentile ²	90 th percentile	95 th percentile	97.5 th percentile
			<u>µg/d</u>	
>25	0.2	5.5	7.0	8.3
>30	2.2	7.8	9.0	10.3
>40	6.3	11.7	13.2	14.5
>50	10.4	15.8	17.3	18.6

¹ Results based on a log-linear model of serum 25(OH)D concentration as a function of vitamin D intake

² The vitamin D intake that will maintain serum 25(OH)D concentrations in 50% of adolescent girls (mean age 11.3y) above the indicated cut-off concentration during winter.

10. Overall summary and conclusions

Background

728. There are two sources of vitamin D in the UK: exposure to sunlight (skin synthesis) and diet. Skin synthesis is the main source of vitamin D for most people.
729. The two major forms of vitamin D are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). Vitamin D₃ is synthesised in the skin of humans and animals by the action of sunlight containing ultraviolet B (UVB) radiation. Vitamin D₂ is the naturally occurring form found in plants and is synthesised by UVB exposure of ergosterol.
730. Dietary sources of vitamin D in the UK are natural food sources, fortified foods and supplements. There are few naturally rich food sources of vitamin D. Those that contain significant amounts are mostly of animal origin and contain vitamin D₃ (e.g., oily fish, red meat, egg yolk). Animal products (e.g., muscle, fat, liver, kidney) also contain the vitamin D metabolite, 25-hydroxyvitamin D. Wild mushrooms are a rich source of vitamin D₂. Vitamin D can also be obtained from foods which are fortified with either vitamin D₂ or D₃ (e.g., breakfast cereals, fat spreads) and from dietary supplements containing vitamin D₂ or D₃. Dietary sources become essential when sunlight containing UVB light is limited (e.g., in winter) or exposure to sunlight containing UVB light is restricted.
731. Dietary reference values (DRVs) for vitamin D, set by the Committee on Medical Aspects of Food Policy (COMA) in 1991 (DH, 1991), were based on prevention of rickets in children and osteomalacia in adults. A dietary intake of vitamin D was not considered necessary for individuals with adequate exposure to sunlight; therefore, a reference nutrient intake (RNI⁷⁸) was not set for individuals aged 4-65 years '*living a normal lifestyle*'. RNI were set only for certain population subgroups considered to be at risk of vitamin D deficiency: infants 0-6 months (8.5 µg/d); infants and children 7 months–3 years (7 µg/d); pregnant and breast-feeding women (10 µg/d), adults aged 65 or more years (10 µg/d), those with limited exposure to sunlight (e.g., confined indoors or wearing concealing clothing) and people of Asian ethnic origin (10 µg/d).
732. The DRVs for vitamin D were reviewed and endorsed by COMA in 1998. Since then, however, studies have suggested a range of non-musculoskeletal health benefits of vitamin D. The data on vitamin D and health outcomes were considered by SACN in 2007. At that time, it was concluded that there was insufficient evidence to amend existing advice and that evidence on the relationship between vitamin D and non-musculoskeletal health was inconclusive.
733. In 2010, SACN agreed to review the DRVs for vitamin D because a substantial amount of data had accumulated since 2007, including a comprehensive report in 2011 by the Institute of Medicine (IOM) in the US⁷⁹, which provided an important resource for consideration of the evidence.

Purpose and scope of current review

734. The purpose of the current SACN review on vitamin D was to consider whether the DRVs for vitamin D set by COMA in 1991 are still appropriate in the context of current lifestyles (e.g., advice to stay out of the sun and to wear sunscreen). The key issues considered were:
- biochemical indicators of vitamin D status and the validity of the values used to assess risk of deficiency and excess;
 - association between vitamin D status and health outcomes at all life stages and the effects of biological modifiers;

⁷⁸ The RNI represents the amount of a nutrient that is likely to meet the needs of 97.5% of the population.

⁷⁹ *Dietary Reference Intakes for Calcium and Vitamin D*.

- the contribution of cutaneous vitamin D synthesis to vitamin D status in the UK taking account of factors that modify skin exposure to sunlight; risks of skin damage and other adverse health outcomes associated with sunlight exposure;
- potential adverse effects of high vitamin D intakes; and
- relative contributions made by dietary vitamin D intake (from natural food sources, fortified foods and supplements) and cutaneous vitamin D synthesis, to the vitamin D status of the UK population.

735. In assessing the evidence on vitamin D and health outcomes, data from RCTs, then prospective studies, were preferred in terms of informing the setting of DRVs when these were available; however, data from other study types were also considered (including case-control, cross-sectional studies and case reports).

Biology & metabolism

736. The first step in endogenous vitamin D synthesis is the conversion by solar UVB radiation of 7-dehydrocholesterol (7DHC) in the skin to previtamin D. The dose response is not linear (12-15% is converted to previtamin D) which means that long exposures do not lead to proportionally greater previtamin D synthesis. Previtamin D₃ is thermodynamically unstable and is converted at body temperature to vitamin D₃ which enters the circulation and is transported to the liver bound to vitamin D binding protein.

737. Dietary vitamin D is lipid soluble and is incorporated into chylomicrons within enterocytes and secreted through the lymph into the systemic circulation where it is transported to the liver.

738. Vitamin D is converted to its active metabolite 1,25-dihydroxyvitamin D (1,25(OH)₂D) in 2 hydroxylation steps: firstly to 25-dihydroxyvitamin D (25(OH)D) in the liver and then to 1,25(OH)₂D in the kidney. 25(OH)D is the major circulating metabolite of vitamin D. Its concentration in serum reflects vitamin D supply from cutaneous synthesis and the diet.

739. Vitamin D accumulates in both adipose tissue and muscle. Details about accumulation and mobilisation of vitamin D from adipose tissue and other tissues such as muscle are not clear at this time.

740. Polymorphisms have been identified in genes encoding proteins involved in vitamin D metabolism which might influence serum 25(OH)D concentrations but the functional relevance of these polymorphisms is not clear.

Biomarkers of exposure

741. The active metabolite of vitamin D, 1,25(OH)₂D, is not a suitable indicator of vitamin D exposure because it is homeostatically regulated and has a short half-life (< 4 hours). Serum 25(OH)D concentration is widely considered to be the best indicator of total vitamin D exposure (from the diet and sunlight) because it has a long half-life in the circulation (about 2-3 weeks) and is not subject to tight homeostatic control. As a marker of exposure to vitamin D, serum 25(OH)D concentration is influenced by those factors that affect dermal synthesis (including season, latitude, clothing, skin type).

742. There are limitations in using serum 25(OH)D concentration as a marker of vitamin D exposure since it has been observed to decrease in response to acute inflammation. It is therefore possible that low serum 25(OH)D concentrations (which have been observed in conditions such as cancer) may simply reflect an underlying inflammatory state. The relationship between exposure and serum 25(OH)D concentration may also be confounded by BMI and genetic variation.

743. Quantification of serum 25(OH)D concentration is influenced by the method used for its measurement. The two main methods in use are antibody or liquid chromatography (LC) based. Most data collected over

the past 20-30 years have been analysed using antibody-based assays. However, LC-based assays which use a tandem mass spectrometer have high specificity, high sensitivity and better reproducibility.

744. The main limitations associated with the methods used for measuring serum 25(OH)D concentration are accuracy and variability. Measurements can vary considerably (15-20%) depending on the type of assay used and across different concentration ranges. There is also lack of agreement between different laboratories using the same methods. This has implications for the interpretation of epidemiological studies and trials that have examined the relationship between serum 25(OH)D concentration and health outcomes.

Photobiology

745. The sun is the main source of ultraviolet radiation (UVR) which is categorised into three types according to wavelength: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm). The UVR spectrum is modified on its path through the atmosphere by ozone, altitude, ground reflection (e.g., by sand or snow), air pollution, cloud cover and shade, time of day and season.

746. The amount of vitamin D synthesised in the skin depends on skin exposure to UVB radiation and efficiency of cutaneous synthesis. Factors that affect skin exposure to UVR include the amount of available sunlight, exposure angle, time spent outdoors, skin coverage and use of sunscreen. The main determinant of sunlight availability is solar elevation which depends on time of year and time of day as well as the weather, which affects outdoor activity and skin coverage.

747. The most effective time for vitamin D synthesis is at midday. In the UK, sunlight is not effective for vitamin D synthesis when the sun is low in the sky (sunrise or sunset) and during winter. Solar radiation is reduced during winter because of low solar elevation, short days and cloudy skies. Solar radiation is greater in the summer because the sun is much higher, the days are longer and the weather less cloudy.

748. There is a well observed seasonal cycle in serum 25(OH)D concentrations in the UK. During winter, the small amount of UVB in sunlight is insufficient to initiate synthesis of any biologically relevant quantities of vitamin D. Sunlight-induced vitamin D synthesis in white-skinned populations becomes effective from March with maximum concentrations observed in September after a summer of exposure. Serum 25(OH)D concentration decreases from October onwards throughout the winter months.

749. UVB (as a proportion of UVR) lessens with increasingly northern latitudes. However, the extent to which increasing latitude reduces vitamin D synthesis is not clear because the weather also gets progressively colder, people go outdoors less and expose less skin.

750. The amount of 7DHC present in the skin decreases with increasing age but the age at which this becomes a limiting factor if there is ample exposure to sunlight is unclear.

751. The pigment melanin absorbs some of the UVB radiation which would otherwise be absorbed by 7DHC. This means that if the absolute dose of UVB radiation is the same as that given to a person with white skin then people with darker skin will synthesise less. However, darker skin has the same capacity to synthesise vitamin D if the dose of radiation is adjusted for the protective effect of melanin.

Vitamin D and health outcomes

752. Vitamin D has been associated with a number of musculoskeletal and non-musculoskeletal health outcomes.

Musculoskeletal health outcomes

753. Evidence on vitamin D and musculoskeletal health outcomes was considered by life stage since different musculoskeletal health measures are appropriate for specific age groups. Rickets was considered in

infants and children; osteomalacia and bone health indices (BMC, BMD, biochemical markers of bone turnover) were considered across all age groups; muscle strength and function was considered in all adults; fracture prevention and risk of falls were considered in adults > 50 y.

754. Evidence on rickets is derived mainly from observational studies and therefore subject to confounding. An important limitation in these studies was that most did not measure calcium intake which is a potential confounding factor in studies on rickets. There was great variation in the serum 25(OH)D concentration at which rickets was present but concentrations were < 25 nmol/L in the majority of studies considered. This suggests that the risk of rickets is increased at serum 25(OH)D concentration < 25 nmol/L; this concentration is, however, not diagnostic of the disease. The concentration below which there is increased risk of rickets specifically due to vitamin D is uncertain.
755. Evidence on vitamin D and osteomalacia is limited and is drawn mainly from case reports. There is no clear serum 25(OH)D threshold concentration below which risk of osteomalacia is increased but concentrations were < 20 nmol/L in all the studies considered.
756. Findings from studies that considered the relationship between vitamin D and bone health indices (BMC/BMD/biochemical markers of bone turnover) varied by life stage. Evidence was suggestive of a positive association between maternal 25(OH)D concentration during pregnancy and bone health indices in the fetus/newborn and of beneficial effects of vitamin D supplementation on bone health indices at some skeletal sites in adults aged > 50 years. Evidence on vitamin D and bone health indices in infants, children and adolescents and adults < 50 years was either inconsistent or insufficient to draw conclusions.
757. Although cohort studies suggest an association between increased serum 25(OH)D concentration and decreased fracture risk, evidence from RCTs show that vitamin D supplements appear to have no effect on fracture risk in older men and women.
758. Evidence on vitamin D and falls is mixed but overall is suggestive of a beneficial effect of vitamin D supplementation in reducing fall risk in community dwelling adults > 50 years with baseline serum 25(OH)D concentrations across a range of values. One study reported an increase in fall risk with vitamin D supplementation (12,500 µg/500,000 IU); however, the dose was very high and administered annually which may produce different effects from daily supplementation.
759. Evidence from RCTs suggest that vitamin D supplementation may improve muscle strength and function in adults < 50 years with mean serum 25(OH)D concentration < 30 nmol/L. In adults > 50 y, the evidence is mixed but, overall, suggestive of beneficial effects on muscle strength and function with mean baseline serum 25(OH)D concentrations across a range of values.

Non-musculoskeletal health outcomes

760. A range of non-musculoskeletal health outcomes were considered: oral health, reproductive health (on maternal & newborn outcomes) all-cause mortality, cancer, cardiovascular disease, hypertension, infectious disease (TB, influenza, respiratory tract infection, obstructive pulmonary disease), autoimmune disease (asthma, atopic disorders, multiple sclerosis, type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis), age-related macular degeneration and neuropsychological functioning (cognitive function, dementia, autism, depression, schizophrenia).
761. Evidence for the proposed benefits of vitamin D on non-musculoskeletal health outcomes is drawn mainly from observational studies so findings might be due to reverse causality (i.e., low 25(OH)D concentration is a consequence of the illness rather than the cause) or confounding by other factors associated with a specific health outcome. There is limited RCT evidence for some non-musculoskeletal health outcomes and the findings are inconsistent.

762. Overall, there was insufficient evidence on vitamin D and non-musculoskeletal health outcomes to inform the setting of DRVs for vitamin D.

Selection of health outcomes to be used as the basis for setting DRVs for vitamin D

763. Musculoskeletal health outcomes (based on evidence relating to rickets, osteomalacia, falls and muscle strength and function) were selected as the basis for setting the DRVs for vitamin D.

764. There was wide variability in the mean and individual serum 25(OH)D concentrations associated with increased risk of poor musculoskeletal health; however, risk appears to increase at concentrations below 20-30 nmol/L. Interpretation of the data is complicated by the fact that measurement of serum 25(OH)D concentration is affected by inter-assay differences. Since various assay methods were used in the studies considered there are difficulties in making comparisons between studies on serum 25(OH)D concentrations associated with risk.

765. The number of uncertainties in the data makes it difficult to identify a specific serum 25(OH)D threshold concentration between 20-30 nmol/L associated with increased risk of poor musculoskeletal health. The current threshold of 25 nmol/L used to define the concentration below which risk of vitamin D deficiency is considered to increase (DH, 1998) is therefore retained. This threshold is not diagnostic of disease but indicative of increased risk of poor musculoskeletal health.

Potential adverse effects of high exposures to vitamin D

766. The endpoint used to assess the effects of high exposure to vitamin D was hypercalcaemia since adverse effects unrelated to elevated calcium have not been reliably documented. The Tolerable Upper Intake Levels (UL⁸⁰) for vitamin D recommended by the European Food Safety Authority (EFSA) are 100 µg/d (4000 IU) for adults and children aged 11-17 y, 50 µg/d (2000 IU) for children aged 1-10 y and 25 µg/d (1000 IU) for infants. The TULs were considered appropriate by the COT⁸¹. The UL may not apply to individuals with some health conditions such as normocalcaemic hyperparathyroidism and granulomatous conditions (including sarcoidosis and tuberculosis) which predispose to hypercalcaemia or to those with genetic predispositions such as idiopathic infantile hypercalcaemia.

767. Case reports of vitamin D toxicity are associated with serum 25(OH)D concentrations > 300 nmol/L and more usually 600-1000 nmol/L. In adults, a single dose of 7500 µg (300,000 IU) vitamin D every 3 months or less would not be expected to result in serum 25(OH)D concentrations > 300 nmol/L but the risk of this occurring in some individuals would be higher with increasing doses. Vitamin D doses of 15000 µg (600,000 IU) would be expected to cause hypercalcaemia and potentially cause adverse effects in infants.

Vitamin D intakes and serum 25(OH)D concentrations in the UK population

768. Nationally representative data on vitamin D intakes and serum/plasma 25(OH)D concentrations of the general population in the UK were drawn from the: National Diet and Nutrition Survey (NDNS) rolling programme of adults and children aged 18 months upwards (2008/09-2011/12); NDNS Scotland report (2008/09-2011/12), 2011 UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC); Health Survey for England (HSE); and Scottish Health Survey (SHS). Data on low income population groups (aged 2+ years; 2003-5) were drawn from the Low income Diet and Nutrition Survey (LIDNS). Data on pregnant women were obtained from UK based cohort studies⁸².

⁸⁰UL - the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. The UL is not a recommended level of intake. It is an estimate of the highest level of intake which carries no appreciable risk of adverse health effects.

⁸¹ Committee on Toxicity of Chemicals in Foods, Consumer Products and the Environment.

⁸² Nationally representative data are not available for pregnant women.

769. Blood collection within the NDNS rolling programme, LIDNS, HSE and SHS is spread evenly across the year. In the DNSIYC blood samples were collected between the months of February and August. In the consideration of these data, it is important to be aware that measurements of serum 25(OH)D concentration from different surveys may not be comparable since they can vary considerably depending on the type of assay used. There is also a lack of agreement between different laboratories using the same methods.
770. The NDNS shows that mean dietary intakes of vitamin D (from food sources) were approximately 7.5-9.8 µg/d for non-breast fed infants aged 4-11 months and 3.5 µg/d for 12-18 months; 2.7-3.2 µg/d for breast fed infants aged 4-11 months and 1.8 µg for those aged 12-18 months; 1.9-2.1 µg/d for ages 1.5-18 years; and 2.8-3.3 µg for 19-65+ years. The mean dietary intakes of vitamin D from all sources (including dietary supplements) were approximately 7.7-10 µg/d for non-breast fed infants aged 4-11 months and 3.9 µg/d for 12-18 months; 3.5-3.8 µg/d for breast fed infants aged 4-11 months and 2.6 µg/d for those aged 12-18 months; 2.3-2.7 µg/d for ages 1.5-18 years; and 3.6-5.1 µg/d for 19-65+ years.
771. For institutionalised adults, mean daily vitamin D intake of men was 3.87µg (39% of RNI) from all sources and 3.79µg (38% of RNI) from food sources; for women, mean daily intake was 3.36µg (34% of RNI) from all sources and 3.31µg (33% of RNI) from food sources.
772. Infant formula was the main contributor to vitamin D intake for infants aged 4-18 months. For all age groups above 18 months of age (except children aged 1.5-3y), meat (and meat products) was the main source of dietary vitamin D providing 23-35% of intake. For children aged 1.5-3y the major contributor to vitamin D intake was milk (and milk products) which provided 24% of intake. Fat spreads (most of which are fortified with vitamin D) and cereals and cereal products contribute 19-21% and 13-20% of intake respectively across all age groups.
773. In the DNSIYC and NDNS annualised⁸³ mean plasma 25(OH)D concentrations in the UK were: 64-69 nmol/L for ages 5-18 months; 58 nmol/L for ages 1.5-3y⁸⁴; 50 nmol/L for ages 4-10 years; 43-45 nmol/L for ages 11-65+y. The percentage with 25(OH)D concentrations < 25 nmol/L was: 5-11 months, 6%; 12-18 months, 2%; 1.5-3 y, 7.5%; 4-10 y, 12-16%; 11-18 y, 20-24%; 19-64 y, 22-24%; 65+ years, 17-24%.
774. For institutionalised adults the mean plasma 25(OH)D concentration was 33.7 nmol/L in men and 32.5 nmol/L in women; 38% of men and 37% of women had a concentration < 25 nmol/L).
775. The HSE and the SHS reported that the percentage of adults (16+ y) with a serum 25(OH)D concentration < 25 nmol/L at any time of year was 20-34% in England and 28-38% in Scotland⁸⁵.
776. For low income groups in the UK, annualised mean plasma 25(OH)D concentrations were 40-44 nmol/L in children aged 11-18 y⁸⁶ and 43-53 nmol/L in adults aged 19-65+ y. The percentage with plasma 25(OH)D concentration < 25 nmol/L was: 8% of boys and 23% of girls aged 11-18 y and 14-25% of adults aged 19-65+ y. In Scotland, mean plasma 25(OH)D concentration was 42 nmol/L in the highest quintile (25% < 25nmol/L) of household income compared with 33 nmol/L in the lowest (42% below 25 nmol/L).
777. For all age groups in the NDNS, mean plasma 25(OH)D concentrations were lowest in the winter months (January-March) and highest in the summer months (July-September). The proportion with plasma 25(OH)D concentration < 25 nmol/L in the winter months was: children (4-10 y), 31%; older children and adolescents (11-18 y), 40%; adults aged 19-64 y, 39%; and adults aged 65+ y, 29%. The proportion with

⁸³ Average of reports from different months of the year.

⁸⁴ Based on small sample size (n=42).

⁸⁵ Although the proportion of adults with a serum 25(OH)D concentration < 25 nmol/L was slightly higher in Scotland than England, there were quality control issues in some of the Scottish data where assay readings had been low.

⁸⁶ Sample size in boys aged 8-10 years was too small (n=8) to be representative.

plasma 25(OH)D concentration < 25 nmol/L in the summer months was: 2% of children (4-10 y); 13% of older children and adolescents (11-18 y); 8% of adults aged 19-64 y; and 4% of adults aged 65+ y.

778. Data from the HSE, SHS and cohort studies show that a proportion of the following population groups do not achieve serum 25(OH)D concentrations of 25 nmol/L in the summer: 17% of adults in Scotland; 16% of adults in London; 53% of women of south Asian ethnic origin in southern England; and 29% of pregnant women in north west London.
779. Nationally representative data are not available for pregnant women, however data from UK based cohort studies suggest mean/medium serum 25(OH)D concentrations in pregnant women of 34-53 nmol/L in summer and 26-34 nmol/L in the winter. The percentage with concentrations < 25 nmol/L ranged from around 25-29% in the summer to 49-76% in the winter.
780. An HSE analysis by ethnicity reported that annualised mean serum 25(OH)D concentration was higher in white adults aged 16+ y (45.8 nmol/L) compared to Asian (20.5 nmol/L) and black (27.7 nmol/L) adults. The 1996 Asian Infant Feeding Survey (age 2 y) reported that the mean serum 25(OH)D concentration (in October-November) was 42 nmol/L in Bangladeshi infants (20% < 25 nmol/L), 36 nmol in Pakistani infants (34% < 25 nmol/L and 42 nmol/L in Indian infants (with 25% < 25 nmol/L). A study in southern England reported that mean serum 25(OH)D concentrations (in every season) was lower in in South Asian women compared to white women and a higher percentage of South Asian women had serum 25(OH)D concentration < 25 nmol/L throughout the year (53% in summer, 80% in autumn, 75% winter, 73% spring) compared with white women (0.4% in summer, 1.8% in autumn, 9.7% winter, 6.7% spring).

Review of DRVs

781. The DRVs describe the distribution of requirements in a population and comprise 3 estimates: the *Estimated Average Requirement (EAR)*, half of a group in a population will need more than this amount and half will need less; the *Reference Nutrient Intake (RNI)*, the amount that will meet the needs of 97.5% of the population; and the *Lower Reference Nutrient Intake (LRNI)*, the intakes which will meet the needs of only 2.5% of the population.
782. Previously in the UK, an RNI for vitamin D was set by COMA only for population groups at high risk of deficiency (DH, 1991) and no EAR or LRNI was set. It was assumed that, for most people, the amount of vitamin D produced by exposure to sunlight containing UVB would be adequate for achieving serum 25(OH)D concentrations above \geq 25 nmol/L during winter. It is now known that this is not the case.
783. In the current review, the health outcome identified as the basis for setting DRVs for vitamin D was musculoskeletal health (based on rickets, osteomalacia, falls and muscle strength). Although the available data were not sufficient to establish a distribution of serum 25(OH)D concentrations that would be required to estimate the 3 DRV estimates for vitamin D intake (i.e., LRNI, EAR, RNI) or a clear threshold serum 25(OH)D concentration to support musculoskeletal health outcomes, the evidence overall suggests that the risk of poor musculoskeletal health is increased at serum 25(OH)D concentrations < 25 nmol/L.
784. A serum 25(OH)D concentration of 25 nmol/L was therefore selected, on a precautionary basis, as the target concentration to protect all individuals from poor musculoskeletal health. This concentration was considered to be a '*population protective level*'; i.e., the concentration that 97.5% of individuals in the UK should be above, throughout the year, in terms of protecting musculoskeletal health.
785. The next step in estimating a DRV for vitamin D was to translate the serum 25(OH)D concentration of 25 nmol/L into an average dietary intake value that would achieve this in 97.5% of the population in the UK and thereby define the RNI for vitamin D. Only an RNI was established because this precautionary protective approach prohibited the establishment of an EAR or LRNI. The average vitamin D intake refers

to the mean or average intake over the long term and takes account of day to day variations in vitamin D intake.

786. Sun exposure is the major source of vitamin D during the summer months for the majority of people in the UK. It was not possible to quantify the sunlight exposure required in the summer months to maintain a winter serum 25(OH)D concentration of at least 25 nmol/L because of the number of factors that affect endogenous vitamin D synthesis, storage and utilisation.
787. The RNI was estimated by modelling data from individual RCTs in adults (men & women, 20-40 y and 64+ y) and adolescent girls (11 y). The RCTs had been conducted in winter so that dermal production of vitamin D was minimal.
788. The modelling exercise indicated that the estimated average daily vitamin D intake required to maintain serum 25(OH)D concentration \geq 25 nmol/L in winter by 97.5% of the population is 12 μ g based on serum 25(OH)D analysis by LC-tandem MS or 9 μ g/d based on analysis of the same sera by immunoassay. Since the target threshold serum 25(OH)D concentration of 25 nmol/L was based on studies which had used a range of different assays to measure serum 25(OH)D concentration, the RNI was set between these 2 estimates, at 10 μ g/d.
789. Data were not available to allow direct determination of the vitamin D intake required to reach serum 25(OH)D concentrations \geq 25 nmol/L in infants and children aged 0-10 y. However evidence suggests that age does not affect the response of serum 25(OH)D concentration to vitamin D intake. Data from the modelling exercise were therefore extrapolated to younger age groups.
790. An RNI of 10 μ g/d is proposed for the UK general population aged 11-64+ y. The RNI assumes minimal sunshine exposure because the studies used to derive this figure were conducted in winter. This is the amount needed to achieve a serum 25(OH)D concentration \geq 25 nmol/L during winter in 97.5% of the population.
791. Although most people would be expected to synthesise vitamin D during summer, serum 25(OH)D concentrations $<$ 25 nmol/L have been observed in a proportion of some population groups in the UK during the summer months (see paragraphs 649-652). Since it is not possible to identify these individuals, it is proposed that the RNI is applicable throughout the year. This is a precautionary approach to protect the most vulnerable groups in the population and to take account of variable exposure to sunshine and diet. This approach ensures coverage of 97.5% of the population throughout the year.
792. The RNI of 10 μ g/d (400 IU) proposed for the general UK population includes pregnant and lactating women.
793. Since data are not available to relate serum 25(OH)D concentration in the infant clearly to current or long term health, *Safe Intakes*⁸⁷ rather than RNIs are proposed for ages 0-3 years. Safe Intakes are based on a precautionary approach and reflect the insecurities of the data. A Safe Intake of 8.5-10 μ g/d (340-400 IU), based on concentrations of vitamin D in infant formula, is proposed for children age 0-11 months. A Safe Intake of 10 μ g/d (400 IU), based on the RNI for the UK population, is proposed for infants and children aged 1-3 years.
794. There is currently no vitamin D RNI for exclusively breast fed infants because it was previously assumed that maternal vitamin D supplementation during pregnancy and breast milk would provide the infant with adequate vitamin D for the period of exclusive breast feeding. The few available data suggest that it is

⁸⁷ COMA (DH, 1991) set a 'Safe Intake' for some nutrients if there were insufficient reliable data to set DRVs. They are set on grounds of prudence and are 'judged to be a level or range of intake at which there is no risk of deficiency, and below a level of where there is a risk of undesirable effects' (DH, 1991).

unlikely that an exclusively breast fed infant in the UK would maintain serum 25(OH)D concentration ≥ 25 nmol/L for 6 months. Therefore the Safe Intakes proposed for non-breast fed infants are also proposed for exclusively breast fed infants (from birth).

795. For ages 4–10 years, the RNI of 10 $\mu\text{g}/\text{d}$ proposed for the UK population (11-64 y) is considered appropriate.
796. Population groups considered to be at risk of having serum 25(OH)D concentrations < 25 nmol/L include people from ethnic groups with dark skin. The role of skin colour, however, is complicated by behaviours that could also affect serum 25(OH)D concentration (e.g., wearing clothes that cover the skin when outdoors; sun avoidance). Other population groups at risk of having individuals with serum 25(OH)D concentrations < 25 nmol/L include frail and institutionalised people and those not spending substantial time outdoors. An increment added to the RNI was not considered necessary for these '*at risk*' population groups because the recommendation that the RNI is applicable throughout the year is to take account of individuals with minimal sunshine exposure, including those most at risk.
797. There is evidence suggesting that obese people are also at risk of low serum 25(OH)D concentrations; however, the data are insufficient to support a different recommendation from that of the general UK population.
798. Although achievement of the proposed RNI by the UK population would lead to an increase in mean/median vitamin D intakes of the UK population, it is unlikely that this would lead to vitamin D intakes at the upper end of the distribution reaching levels that might pose a risk of adverse effects.

11. Recommendations

799. Serum 25(OH)D concentration is an indicator of exposure to vitamin D (i.e., from the diet and skin synthesis). In order to protect musculoskeletal health, it is recommended that the serum 25(OH)D concentration of individuals in the UK should not fall below 25 nmol/L at any time of the year.
800. In the UK, population groups at increased risk of having a serum 25(OH)D concentration < 25 nmol/L are those with minimal sunshine exposure as a result of not spending substantial time outdoors (e.g., frail and institutionalised people) or due to the habitual wearing of clothing that covers most of the skin while outdoors.
801. It is not possible to make a recommendation regarding the amount of sunlight exposure that would be required during the summer to maintain serum 25(OH)D concentration \geq 25 nmol/L in 97.5% of the population during winter because of the number of factors that affect endogenous vitamin D production.
802. A Reference Nutrient Intake (RNI) for vitamin D of 10 $\mu\text{g}/\text{d}$ is therefore proposed for the UK population aged 4 years and over. This is the amount needed for 97.5% of the population to maintain a serum 25(OH)D concentration of 25 nmol/L when UVB sunshine exposure is minimal.
803. The RNI of 10 $\mu\text{g}/\text{d}$ proposed for the whole UK population includes individuals from minority ethnic groups with darker skin.
804. It is proposed that the RNI is applicable throughout the year, as a precautionary measure, to cover population groups in the UK identified to be at risk of minimal sunshine exposure as well as unidentified individuals in the population with minimal sunshine exposure who would be at risk of 25(OH)D concentrations < 25 nmol/L in summer.
805. Data are insufficient to set RNIs for infants and children aged 0-3 years. As a precaution, a 'Safe Intake' of vitamin D is therefore proposed for these ages: in the range 8.5-10 $\mu\text{g}/\text{d}$ for ages 0 to < 1 year (including exclusively breast fed infants); and 10 $\mu\text{g}/\text{d}$ for ages 1 to < 4 years.
806. Since it is difficult to achieve the RNI/Safe Intake from natural food sources alone, it is recommended that consideration is given to strategies for the UK population to achieve the RNI of 10 $\mu\text{g}/\text{d}$ for those aged 4 years and older and for younger children to achieve a *Safe Intake* in the range 8.5-10 $\mu\text{g}/\text{d}$ at ages 0 to < 1 year and 10 $\mu\text{g}/\text{d}$ at ages 1 to < 4 years.

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