Vitamin D and Health

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Scientific Advisory Committee on Nutrition

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Preface

The Scientific Advisory Committee on Nutrition (SACN) previously considered the evidence on vitamin D and health in 2007. It concluded that there were insufficient data at that time to reconsider the Dietary Reference Values (DRVs) for vitamin D set by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) in 1991. Since vitamin D is synthesised in the skin when it is exposed to sunlight, COMA considered that dietary intakes of vitamin D were not necessary for most of the UK population (aged 4-64y). It was assumed that skin synthesis of vitamin D in the summer would be enough to cover requirements during the winter. Reference Nutrient Intakes (RNI) were therefore set only for groups considered at risk of vitamin D deficiency.

In 2010, SACN agreed to consider whether the DRVs for vitamin D intake were still appropriate in the context of public health advice to stay out of the sun and to wear sunscreen and because a substantial amount of new evidence had accumulated since its previous considerations.

In a change to previous advice, SACN is now recommending an RNI for vitamin D of 10 µg/d (400 IU/d), throughout the year, for everyone in the general UK population aged 4y and above. The RNI of 10 µg/d (400 IU/d) for the general UK population includes pregnant and lactating women and population groups at increased risk of vitamin D deficiency. Since there were insufficient data to set RNIs for children aged under 4y, Safe Intakes[1] are being recommended for this age group (8.5-10 µg/340-400 IU per day for all infants aged under 1y and 10 µg/400 IU per day for ages 1 up to 4y). The RNI/Safe Intakes have been developed to ensure that the majority of the UK population has a satisfactory vitamin D status (as measured in the blood) throughout the year, in order to protect musculoskeletal health. It was not possible to quantify and take account of sunlight exposure in setting the DRVs because of the number of factors that affect endogenous vitamin D synthesis.

Since it is difficult to achieve the RNI/Safe Intakes from natural food sources alone, SACN is also recommending that the Government considers strategies to help the UK population consume the recommended intakes of vitamin D.

I would like to thank all those individuals and organisations who provided comments on the draft version of this report during the public consultation. All the comments were carefully considered before the report was finalised and the process assisted SACN in refining and clarifying the text.

Completion of this report has been a long and challenging task for SACN because consideration of the evidence was complicated by the fact that vitamin D is obtained from sunlight exposure as well as from food and supplements. I want to thank the members of the SACN Working Group on Vitamin D for their continuing commitment and their contributions to the report, especially the Chair, Professor Hilary Powers, and the secretariat. I also want to thank Professor Ann Webb (Manchester University) and Professor Antony Young (King's College, London) for providing helpful advice and information on the photobiology of vitamin D.

Dr Ann Prentice
Chair, Scientific Advisory Committee on Nutrition
2016

[1] Safe Intakes were set by COMA if there were insufficient reliable data to set DRVs.

Membership of Scientific Advisory Committee on Nutrition: Working Group on Vitamin D

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Professor Roger Francis	Consultant Physician, Metabolic Bone Service, The Newcastle upon Tyne Hospitals.
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Professor Harry McArdle	SACN member; Professor of Biomedical Sciences, University of Aberdeen; Honorary Professor of Biomedical Sciences, University of Nottingham.
Dr Ann Prentice	Chair of SACN; Director, MRC Human Nutrition Research, Cambridge.
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Dr Anthony Williams	SACN member; Formerly, Reader in Child Nutrition and Consultant in Neonatal Paediatrics, St George's, University of London.
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Ms Emma Peacock (Scientific) *(February 2013 - December 2015)*

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Mr Heiko Stolte (Scientific) *(until January 2013)*

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Chair

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Members

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Ms Christine Gratus (until March 2013)	Lay representative; retired advertising and marketing research director.
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Ms Gemma Paramor	Lay representative; finance professional in accounting and fund management.
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Dr Stella Walsh	Consumer representative.
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Professor Ian Young	Professor of Medicine, Queen's University Belfast.

Summary

Background

S.1 Vitamin D is required for regulation of calcium and phosphorus metabolism and is therefore important for musculoskeletal health. It is synthesised in the skin upon exposure to sunlight containing sufficient ultraviolet B (UVB) radiation and this is the main source for most people. It can also be obtained from foods or dietary supplements. Dietary sources are essential when sunlight containing UVB radiation is limited (e.g., during the winter months) or exposure to it is restricted (e.g., due to lack of time spent outdoors or little skin exposure).

S.2 Dietary Reference Values (DRVs) for vitamin D were set by the Committee on Medical Aspects of Food Policy (COMA) in 1991 and were based on prevention of rickets in children and osteomalacia in adults. A Reference Nutrient Intake (RNI)[2] for vitamin D was not set for individuals (aged 4-64 y) with regular exposure to sunlight because it was considered that enough vitamin D would be synthesised in the summer to cover their needs in the winter. RNIs for vitamin D (7-10 µg/280-400 IU per day) were set only for UK population groups considered to be at risk of vitamin D deficiency: infants (0-3 y); pregnant and breast-feeding women; adults age 65y and above; those with limited sunlight exposure; and women and children of Asian ethnic origin. The DRVs were reviewed and endorsed by COMA in 1998.

S.3 Although the DRVs were based on bone health, emerging evidence has also suggested a range of other health benefits of vitamin D. In 2007, the Scientific Advisory Committee on Nutrition (SACN) concluded that there was insufficient evidence at that time to warrant reviewing the DRVs for vitamin D set by COMA and that evidence on vitamin D and non-musculoskeletal health was inconclusive. In 2010, SACN agreed to review the DRVs for vitamin D because a substantial amount of published data had accumulated since its previous considerations.

Terms of reference

S.4 The terms of reference were: *to review the Dietary Reference Values for vitamin D and make recommendations*. This required a risk assessment of the vitamin D status of the UK population and consideration of the following:

a) biochemical indicators of vitamin D status and the validity of the values used to assess risk of deficiency and excess;

b) association between vitamin D status and health outcomes at different life stages and different population groups and the effects of biological modifiers;

c) 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; risks of skin damage and other adverse health outcomes associated with sunlight exposure;

d) potential adverse effects of high vitamin D intakes;

e) 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.

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

Metabolism

S.5 Vitamin D is converted to its active metabolite, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), in two hydroxylation steps. The first hydroxylation is in the liver, where vitamin D is converted to 25-hydroxyvitamin D (25(OH)D), which is the major circulating metabolite of vitamin D and is widely used as a biomarker of vitamin D status; the second hydroxylation is in the kidney where 25(OH)D is converted to $1,25(OH)_2D$.

Photobiology

S.6 Vitamin D is synthesised endogenously by the action of sunlight containing UVB radiation, which converts 7-dehydrocholesterol (7-DHC) in the epidermis to previtamin D followed by thermal isomerisation to vitamin D. Exposure of skin to UVB radiation is influenced by many factors; these include time of day, season, latitude, altitude, cloud cover, air pollution, clothing and sunscreen use.

S.7 At latitudes below 37°N, UVB radiation is sufficient for year round vitamin D synthesis. At higher latitude, vitamin D is not synthesised during the winter months. In the UK, sunlight-induced vitamin D synthesis is only effective between late March/early April and September and not from October onwards throughout the winter months.

S.8 Lower plasma/serum 25(OH)D concentrations have been observed in people with dark skin pigmentation compared to those with lighter skin colour. It is not clear, however, if this is due to skin pigmentation or to physiological or lifestyle differences since dark skin is only one of many cultural and biological factors that could influence the plasma/serum 25(OH)D concentration of individuals from ethnic groups with darker skin pigmentation.

S.9 Efficiency of cutaneous vitamin D synthesis may be lower in people with dark skin and in older people but the evidence is limited.

Biomarkers of vitamin D exposure

S.10 Plasma/serum 25(OH)D concentration is widely used as a biomarker of vitamin D status because it reflects vitamin D supply from cutaneous synthesis and diet but also because it has a long half-life in the circulation (about 2-3 weeks) and is not under tight homeostatic control. A limitation of its use is that it has been observed to decrease in response to acute inflammation, so low concentrations (e.g., observed in conditions such as cancer) may reflect an underlying inflammatory state. The relationship between vitamin D exposure and serum 25(OH)D concentration may also be influenced by Body Mass Index and genetic variation.

S.11 There are also limitations associated with the methods used for measurement of serum 25(OH)D concentration, since measurements can vary considerably (15-20%) depending on the type of assay used. In addition, there is considerable variation between different laboratories using the same methods. These limitations have implications for interpretation of studies that have examined the relationship between serum 25(OH)D concentration and health outcomes.

Vitamin D and health outcomes

S.12 The purpose of reviewing data on vitamin D and a range of health outcomes was to assess whether they might inform the setting of DRVs for vitamin D. In assessing the evidence, data from randomised controlled trials (RCTs), then prospective studies, were preferred in terms of informing the setting of DRVs; however, evidence from other study types was also considered.

S.13 For each of the potential health outcomes considered, a judgement was made on whether the evidence suggested a relationship with vitamin D supplementation or serum 25(OH)D concentration. If the evidence was suggestive of a relationship then the data were examined further to assess whether a range of serum 25(OH)D concentrations or a threshold serum 25(OH)D concentration associated with beneficial effects could be identified. An important limitation to this task 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 varied across studies and were predefined according to different criteria for deficiency. As a consequence, the selected cut-offs were insecure and made it difficult to assess if there was a dose-response relationship.

Musculoskeletal health outcomes

Rickets

S.14 Evidence was mainly from cross-sectional observational studies and case reports and may therefore have been influenced by confounding. Since most studies did not measure calcium intake it was not clear whether the cause of rickets was vitamin D deficiency and/or calcium deficiency. A distinct threshold serum 25(OH)D concentration above which there is no risk of rickets could not be identified but the data suggested overall that the risk increased at serum 25(OH)D concentration < 25 nmol/L; this concentration is, however, not a clinical threshold diagnostic of the disease.

Osteomalacia

S.15 Evidence was limited mainly to case reports. There was no clear serum 25(OH)D threshold concentration below which risk of osteomalacia increased but individual concentrations were < 20 nmol/L in case reports and mean concentrations were ≤ 15 nmol/L in cross-sectional analyses.

Bone health indices (bone mineral content, bone mineral density, biochemical markers of bone turnover)

S.16 Findings varied by life stage. Evidence suggested a positive association between maternal serum 25(OH)D concentration during pregnancy and bone health indices in the fetus/newborn and beneficial effects of vitamin D supplementation on bone health indices in adults aged ≥ 50y. Effects of vitamin D supplementation on bone health indices in infants, children, adolescents and adults < 50y were inconsistent. The evidence base for children (1-3y) and adults < 50y was insufficient to draw conclusions.

Fracture prevention

S.17 Data in adults ≥ 50y are mixed but, on balance, suggest that vitamin D supplementation does not reduce fracture risk. The evidence base on the effect of vitamin D supplementation on stress fracture risk on adults < 50y was insufficient to draw conclusions.

Muscle strength and function

S.18 Limited evidence suggested a beneficial effect of vitamin D supplementation on muscle strength and function in adolescents and adults < 50y with a pre-intervention mean serum 25(OH)D concentration < 20 nmol/L and < 30 nmol/L respectively. For adults ≥ 50 y, with mean baseline serum 25(OH)D concentrations across a range of values, the evidence was mixed but overall suggested that vitamin D supplementation improves muscle strength and function.

Falls

S.19 Evidence was mixed but, overall, suggested vitamin D supplementation reduces fall risk in community dwelling adults ≥ 50y with mean baseline serum 25(OH)D concentrations across a range of values.

Two studies reported an increase in fall risk with vitamin D supplementation; however, doses were very high and administered annually[3] or monthly[4] which may produce different effects from daily supplementation at lower doses.

Non-musculoskeletal health outcomes

S.20 Non-musculoskeletal health outcomes considered were: reproductive health (on maternal & newborn outcomes), cancer, cardiovascular disease, hypertension, all-cause mortality, immune modulation, infectious diseases, neuropsychological functioning, oral health and age-related macular degeneration.

S.21 Evidence on vitamin D and non-musculoskeletal health outcomes is drawn mainly from observational studies so findings of beneficial effects could be due to reverse causality or confounding by other factors associated with a specific health outcome. Results from RCTs of vitamin D supplementation are inconsistent.

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

S.22 Data on vitamin D and non-musculoskeletal health outcomes were considered insufficient to inform the setting of DRVs for vitamin D. Musculoskeletal health (based on rickets, osteomalacia, falls and muscle strength and function) was selected as the basis for setting the DRVs for vitamin D.

S.23 Studies on musculoskeletal health outcomes suggesting beneficial effects of vitamin D (rickets, osteomalacia, falls, muscle strength & function) were considered further to assess whether a range or threshold serum 25(OH)D concentration associated with beneficial effects could be identified. With the exception of case reports, most studies considered provided only mean/median serum 25(OH)D concentrations of participants so it was not possible to establish a range of serum 25(OH)D concentrations associated with the selected musculoskeletal health outcomes.

S.24 There were many uncertainties in the data and wide variability in the mean and individual serum 25(OH)D concentrations associated with increased risk of rickets, osteomalacia and falls or improvement in muscle strength and function. However, the evidence overall suggested that risk of poor musculoskeletal health was increased at serum 25(OH)D concentrations below about 20-30 nmol/L. It was not possible to identify a *specific* serum 25(OH)D threshold concentration between 20-30 nmol/L associated with increased risk of poor musculoskeletal health since various assay methods were used in the studies considered and measurement is influenced by the analytical methodology. The current threshold of 25 nmol/L, used to define the concentration below which risk of vitamin D deficiency increases, was therefore retained. This is not a clinical threshold diagnostic of disease but indicative of increased risk of poor musculoskeletal health.

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

Vitamin D intakes

S.25 Mean dietary intakes of vitamin D from all sources (including supplements) were: 8-10 µg/d (320-400 IU/d) and 3.5 µg/d (140 IU/d) for non-breastfed infants aged 4-11m and 12-18m respectively; 2-3 µg/d (80-120 IU/d)for breast fed infants aged 4-18m; 2-4 µg/d (80-160 IU/d) for ages 1.5-64y; 5 µg/d (200 IU/d) for adults aged ≥ 65y and 3-4 µg/d (120-160 IU/d) for institutionalised adults aged ≥ 65y.

[3] 12,500 µg/500,000 IU.
[4] 1500 µg/60,000 IU or 600 µg/24,000 IU vitamin D_3 + 300 µg 25(OH)D_3.

Plasma/serum 25(OH)D concentration

S.26 Annualised[5] mean plasma 25(OH)D concentrations across the different age groups in the UK ranged between 40 and 70 nmol/L but were lower for institutionalised adults (around 30 nmol/L).

S.27 The proportion of the population (by age) with a plasma 25(OH)D concentration < 25 nmol/L was: 2-8%, 5m-3y; 12-16%, 4-10y; 20-24%, 11-18y; 22-24%, 19-64y; 17-24%, ≥ 65y and above. Nearly 40% of institutionalised adults had a plasma 25(OH)D concentration < 25 nmol/L.

S.28 For all age groups in the UK, mean plasma 25(OH)D concentration was lowest in winter and highest in summer. Around 30-40% of the population had a plasma 25(OH)D concentration < 25 nmol/L in winter compared to 2-13% in the summer. A large proportion of some population groups did not achieve a plasma/serum 25(OH)D concentration ≥ 25 nmol/L in 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 NW London).

S.29 Analysis by ethnicity showed that the annualised mean serum 25(OH)D concentration was higher in white adults aged ≥ 16y (45.8 nmol/L) compared to Asian (20.5 nmol/L) and black (27.7 nmol/L) adults.

Review of DRVs

S.30 Evidence suggests that the risk of poor musculoskeletal health is increased at serum 25(OH)D concentrations below 25 nmol/L. This concentration therefore represents a *'population protective level'*; i.e., the concentration that individuals in the UK should be above, throughout the year, in terms of protecting musculoskeletal health.

S.31 It was not possible to quantify the sunlight exposure that would be required in the summer to achieve a winter serum 25(OH)D concentration ≥ 25 nmol/L because of the number of factors that affect cutaneous vitamin D synthesis.

S.32 The RNI for vitamin D was therefore derived by estimating the average vitamin D intake that would be required for individuals in the UK to achieve a serum 25(OH)D concentration ≥ 25 nmol/L. The average vitamin D intake refers to average intake over the long term and takes account of day to day variations in vitamin D intake.

S.33 The RNI was estimated by modelling data from individual RCTs conducted in winter (so that cutaneous vitamin D synthesis arising from current UVB exposure was minimal) with adults (20-40y & ≥ 64y) and adolescent girls (11 y). The average daily vitamin D intake required to maintain serum 25(OH)D concentration ≥ 25 nmol/L in winter by the majority (97.5%) of the population was estimated to be around 10 µg (400 IU). Data from these RCTs were extrapolated to younger age groups.

S.34 Data were not available to relate serum 25(OH)D concentration in the infant clearly to current or long term health. S*afe Intakes*[6] rather than RNIs were therefore recommended for infants and children aged under 4y in the range of 8.5-10 µg/d (340-400 IU/d).

[5] Average of reports from different months of the year.

[6] COMA (DH, 1991) set a *'Safe Intake'* for some nutrients if there were insufficient reliable data to set DRVs. They 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).

Recommendations

S.35 Serum 25(OH)D concentration is an indicator of exposure to vitamin D (from skin synthesis and dietary intake). In order to protect musculoskeletal health, it is recommended that the serum 25(OH)D concentration of all individuals in the UK should not fall below 25 nmol/L at any time of the year.

S.36 In the UK, individuals in 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 time outdoors (e.g., frail and institutionalised people) or habitually wearing clothing that covers most of the skin while outdoors and those from minority ethnic groups with dark skin.

S.37 It is not possible to make any recommendations regarding the amount of sunlight exposure that would be required during the summer to maintain serum 25(OH)D concentration ≥ 25 nmol/L in 97.5% of the UK population during the following winter because of the number and complexity of factors that affect endogenous vitamin D production.

S.38 An RNI for vitamin D, of 10 µg/d (400 IU/d), is recommended for the UK population aged 4y and above. This is the average amount needed by 97.5% of the population to maintain a serum 25(OH)D concentration ≥ 25 nmol/L when UVB sunshine exposure is minimal. It refers to average intake over a period of time (e.g., a week) and takes account of day to day variations in vitamin D intake.

S.39 The RNI of 10 µg/d (400 IU/d) proposed for the general UK population (aged 4y and above) includes pregnant and lactating women and population groups at increased risk of having a serum 25(OH)D concentration < 25 nmol/L. A separate RNI is not required for these groups. This is a change from previous advice.

S.40 Data are insufficient to set RNIs for infants and children aged under 4y. As a precaution, a 'Safe Intake' of vitamin D is recommended for these ages: in the range 8.5-10 µg/d (340-400 IU/d) for ages 0 up to 1y (including exclusively breast fed and partially breast fed infants, from birth); and 10 µg/d (400 IU/d) for ages 1 up to 4y. The recommendation for exclusively breast fed infants is a change from previous advice.

S.41 It is recommended that the RNI/Safe Intakes are applicable throughout the year, as a precautionary measure, to cover population groups in the UK identified to be at risk of having a serum 25(OH)D concentration < 25 nmol/L (see paragraph S.36 above) as well as unidentified individuals in the population at risk of having a serum 25(OH)D concentration < 25 nmol/L in summer.

S.42 The RNI/Safe Intake for vitamin D refers to intakes from *all* dietary sources: natural food sources; fortified foods (including infant formula milk); and supplements. Since it is difficult to achieve the RNI/Safe Intake from natural food sources alone, it is recommended that the Government gives consideration to strategies for the UK population to achieve the RNI of 10 µg/d (400 IU/d) for those aged 4y and above and for infants and younger children to achieve a Safe Intake in the range 8.5-10 µg/d (340-400 IU/d) at ages 0 to < 1y and 10 µg/d (400 IU/d) at ages 1 to < 4y.

1. Introduction

Background

1.1 Vitamin D is synthesised in the skin by the action of sunlight containing ultraviolet B (UVB) radiation. 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.

1.2 The Committee on Medical Aspects of Food and Nutrition Policy (COMA) set Dietary Reference Values (DRVs) for vitamin D in 1991 (DH, 1991). COMA did not set a Reference Nutrient Intake (RNI[7]) for groups in the population considered to receive adequate sunlight exposure because it was assumed that, for most people, the amount of vitamin D produced by exposure to UVB radiation in the summer would provide enough for their needs during winter.

1.3 Current UK Government advice is that a dietary intake of vitamin D is not 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/400 IU), infants and children aged under 4y (7-8.5 µg/17.5-21 IU); adults aged 65y or above (10 µg/400 IU); those with limited exposure to the sun (e.g., if they cover their skin for cultural reasons or are housebound) (10 µg/400 IU) and women and children of Asian origin (10 µg/400 IU). The DRVs for vitamin D were reviewed and endorsed by COMA in 1998 (DH, 1998).

1.4 Although the current recommendations for vitamin D are based on bone health, it has been suggested that vitamin D may have a role in other health outcomes, which include reducing the risk of cancers, cardiovascular disease (CVD), infectious diseases and autoimmune diseases.

1.5 The evidence on vitamin D and health was previously considered by the Scientific Advisory Committee on Nutrition (SACN) in 2007 (SACN, 2007). In its position statement '*Update on Vitamin D'* SACN concluded that there was insufficient evidence, at that time, 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.

1.6 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* (IOM, 2011)[8]; and numerous studies on vitamin D and various health outcomes.

Terms of Reference

1.7 The *SACN Working Group on Vitamin D* (WG) 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 the sun and to wear sunscreen).

[7] The amount of a nutrient that is sufficient to meet the needs of 97.5% of the population.
[8] Draft report published in 2010.

1.8 The terms of reference were: *to review the Dietary Reference Values for vitamin D and make recommendations.* This required a risk assessment of the vitamin D status of the UK population and consideration of the following:

a) biochemical indicators of vitamin D status and validity of the values used to assess risk of deficiency and excess;

b) association between vitamin D status and various health outcomes at different life stages and in different population groups and the effects of biological modifiers;

c) 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;

d) potential adverse effects of high vitamin D intakes;

e) 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

SACN's remit

1.9 SACN's remit is to assess the risks and benefits of nutrients/foods to health by evaluating published scientific evidence and, based on its assessment, make dietary recommendations for the UK. SACN's dietary recommendations are applicable to the UK general healthy population, including any vulnerable (*at risk*) groups which have been identified. They are not intended as guidance for clinical practice and are not applicable to individual patient care. Consideration of the evidence base is therefore restricted largely to studies in healthy populations that examine the role of a particular nutrient/food in disease prevention rather than cure.

1.10 Before providing advice, SACN assesses possible risks that may be associated with implementing particular recommendations; e.g., potential risks of excess intakes or adverse impacts on other health outcomes. In addition, areas of uncertainty are identified and form the basis of recommendations for further research. SACN's remit does not include providing advice on strategies for implementation of its recommendations; i.e., the committee's role is risk assessment and not risk management.

Scope of report

1.11 Key issues considered in this report are: biology, metabolism and photobiology of vitamin D; measurement of vitamin D exposure (from diet and sunlight), the relationship between vitamin D exposure and markers of vitamin D status; health outcomes associated with vitamin D deficiency and excess; and dietary vitamin D intakes and vitamin D status of the UK population. The potential contribution of sunlight exposure and diet to the vitamin D status of the UK population was also explored.

1.12 The WG's review of the evidence on vitamin D included consideration of the risks associated with sunlight exposure. Although the WG considered the contribution made by sunlight exposure to cutaneous vitamin D synthesis, its remit did not include a review of other benefits of sunlight exposure.

1.13 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 DRVs for vitamin D. The health outcomes examined were those considered to be of public health relevance:

- Musculoskeletal health outcomes - rickets, osteomalacia, bone health indices (e.g. bone mineral content, bone mineral density, biochemical markers of bone turnover), fracture prevention, risk of falls and muscle health.
- Non-musculoskeletal health outcomes - effect of vitamin D during pregnancy and lactation on non-skeletal outcomes in mother & baby, cancers, CVD & hypertension, all-cause mortality, immune modulation, infectious diseases, neuropsychological functioning, oral health and age-related macular degeneration.

Process for consideration of the evidence

1.14 The IOM report (2011) provided an important and comprehensive database and a useful reference resource for consideration of the evidence on vitamin D and health outcomes. In order to inform its 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) and AHRQ-Tufts (Chung et al., 2009). The IOM synthesised the evidence from the Ottawa and Tufts reviews and conducted its own literature search to update the AHRQ reviews. In 2014, the AHRQ published an update of studies conducted since its 2009 review (Newberry et al., 2014). In general, the main findings of the update did not differ from those of the earlier review (Chung et al., 2009) or the IOM update of the evidence.

1.15 The WG considered the data included in the IOM report together with evidence published since then including findings from studies identified in the AHRQ update (Newberry et al., 2014) .

1.16 In this review, 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 evidence published since the IOM report. The position papers, and the original studies cited in the position papers, provided the basis for discussions and judgements on the quality of the evidence. Evidence published up to March 2016 was considered.

1.17 Evaluation of the evidence was based on SACN's *Framework for the Evaluation of Evidence*[9] 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 type has its own strengths and weaknesses. In general, most weight is placed on randomised controlled trials (RCTs) since this is the only study type that can demonstrate a causal relationship between a particular intervention and a health outcome. Less weight is placed on observational (non-intervention) studies because such studies can only show an association between an exposure and a health outcome. In addition, 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).

[9]Last updated, May 2012. Available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/480493/SACN_Framework_for_the_Evaluation_of_Evidence.pdf

1.18 In assessing the evidence on vitamin D and health outcomes, data from RCTs, followed by prospective studies, were preferred in terms of informing the setting of DRVs. However, the portfolio of evidence that was considered comprised some data from other study types including case-control and cross-sectional studies and case reports. Within in each study type, systematic reviews/meta-analyses were considered first. Individual studies that contributed to or were published subsequent to the systematic reviews/meta-analyses were then considered. Where more than one systematic review/meta-analysis addressed the same health outcome, there was sometimes overlap in the primary data selected for inclusion.

1.19 Consideration of the evidence base was restricted mainly to studies in healthy populations that examined whether vitamin D reduced the risk of specific health outcomes and not its effect as a therapeutic agent in reducing severity or progression of pre-existing disease. However, this was not possible in considering the relationship between vitamin D and rickets and osteomalacia since the evidence was drawn mainly from case reports of individuals who presented with symptoms of disease.

1.20 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. Advice on adverse effects of high vitamin D intakes was provided by the *Committee on toxicity of chemicals in food, consumer products and the environment* (COT). Information on the photobiology of vitamin D was provided by Professor Antony Young (King's College, London) and Professor Ann Webb (University of Manchester).

1.21 The draft report was made available for public consultation and the comments received from interested parties were taken into consideration before the report was finalised. The working procedures for the preparation and finalisation of the report are described in Annex 1.

2. Biology and metabolism

2.1 Vitamin D plays an important role in the regulation of calcium and phosphorus metabolism and, therefore, in bone health (Jones et al., 1998).

2.2 Vitamin D is synthesised in the skin by the action of sunlight containing 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).

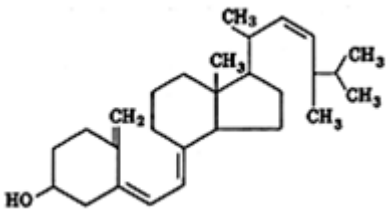
2.3 The two major forms of vitamin D are vitamin D_3 (also referred to as cholecalciferol) and vitamin D_2 (also referred to as ergocalciferol). In this report, the term vitamin D refers to both vitamin D_3 and D_2 unless the specific form is indicated.

Chemistry

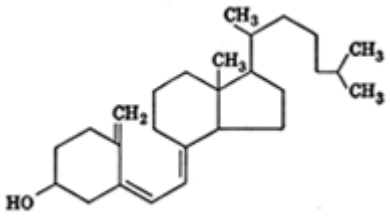
2.4 Vitamin D is classified as a secosteroid. Vitamin D_2 ($C_{28}H_{44}O$) differs structurally from vitamin D_3 ($C_{27}H_{44}O$) in the side chain attached to the secosteroid skeleton, which contains an additional methyl group on carbon atom 24 and a double bond between carbon atoms 22 and 23 (Norman, 2008) (see Figure 1). This difference means that the molecular mass of vitamin D_2 (396.65 g/mol) is 3.1% higher than that of vitamin D_3 (384.64 g/mol).

FIGURE 1: Molecular structure of vitamins D_2 and D_3

Vitamin D_2



Vitamin D_3



Units of measurement and terminology

2.5 Vitamin D intake is expressed in International Units (IU) or in micrograms (µg). IUs are based on antirachitic activity[10] measured in bioassays using rats. One IU of vitamin D is defined by the World Health Organization (WHO) as the activity produced by 0.025 µg of crystalline vitamin D_3 (WHO, 1950); 1 µg of vitamin D3 is equivalent to 40 IU. Although this definition is based on vitamin D_3 activity, the conversion continues to be generalised to both forms of the vitamin regardless of the difference in their molecular mass.

2.6 Serum/plasma concentration of 25-hydroxyvitamin D [25(OH)D], which is the major circulating metabolite of vitamin D, is 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,

[10] The amount required to prevent rickets.

there is not absolute correspondence in the conversion of ng to nmol for 25(OH)D_2 and 25(OH)D_3. The inconsistencies relating to the measurement of the two forms of vitamin D need to be considered in the interpretation of studies comparing vitamin D_2 and D_3.

2.7 In this report:

- the unit of measurement used to express intake is µg; the corresponding amount in IUs is also provided;
- the unit used to express serum/plasma 25(OH)D concentration is nmol/L;
- the term *plasma* is used when describing physiological events; the terms *serum* or *plasma* are used as reported in specific studies; otherwise, for simplicity, the term *serum* is used.

Sources

2.8 Vitamin D is obtained by cutaneous synthesis and from foods or dietary supplements containing either vitamin D_2 or D_3. Vitamin D_3 is the only form produced cutaneously. Vitamin D_3 has also been identified in some plants (see paragraph 2.15 below). Vitamin D_2 is formed in fungi and yeast by UVB exposure of the steroid, ergosterol (a cell membrane component of fungi) and small amounts are present in plants contaminated with fungi.

2.9 A metabolite of vitamin D, 25(OH)D (see paragraphs 2.30-2.31), is present in animal products.

Cutaneous synthesis

2.10 Vitamin D_3 is produced endogenously from 7-dehydrocholesterol (7-DHC) 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 7-DHC in the epidermis is converted to previtamin D_3, which reaches a maximum concentration in the skin within a few hours (Holick et al., 1980) . Even with prolonged irradiation in sunlight the amount of previtamin D formed is limited to 12-15% of the original 7-DHC (MacLaughlin et al., 1982; Webb et al., 1988).

2.11 Previtamin D_3 is thermodynamically unstable. It is converted to the more stable vitamin D_3 in an uncatalysed temperature-dependent isomerisation reaction which takes place in the plasma membrane of epidermal cells over a period of 2-3 days (Holick et al., 1980; MacLaughlin et al., 1982). Prolonged UVB exposure results in conversion of previtamin D_3 to lumisterol and tachysterol which are biologically inactive (Holick et al., 1981). Cutaneous vitamin D_3 can also undergo photoconversion and isomerise into a variety of photoproducts including suprasterol I, suprasterol II and 5,6 transvitamin D_3 (Webb et al., 1989). These photoconversions, which are reversible if concentrations of previtamin D_3 fall, prevent accumulation of toxic amounts of vitamin D_3 from cutaneous exposure alone (Holick et al., 1980).

2.12 The amount of vitamin D_3 made in the skin depends on exposure of the skin to UVB radiation and efficiency of cutaneous synthesis (Holick, 2005; Webb, 2006). 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 may be lower in people with darker skin pigmentation (Clemens et al., 1982) and in older people (MacLaughlin & Holick, 1985) but the evidence is limited. Cutaneous synthesis of vitamin D, factors affecting its production as well as adverse effects of sunlight exposure are considered further in chapter 3.

Dietary sources

2.13 In the UK, the main dietary sources of vitamin D are foods of animal origin, fortified foods and supplements. Commercial synthesis of vitamin D_3 and D_2 is by UVB irradiation of 7-DHC (from sheep wool) and ergosterol (from fungi) respectively (Bikle, 2009).

2.14 There are few naturally rich food sources of vitamin D. Foods that contain significant amounts are mostly of animal origin and contain vitamin D_3. Rich sources include egg yolk (12.6 µg/504 IU per 100 g) and oily fish (5-16 µg/200-640 IU per100 g) such as salmon, mackerel, herring and sardines (Finglas et al., 2015). Animal products such as meat, fat, liver and kidney also contain vitamin D_3 (0.1-1.5 µg/4-60 IU per 100 g), as well as the vitamin D metabolite 25(OH)D_3 (Ovesen et al., 2003).

2.15 Vitamin D_3 and 7-DHC have also been identified in the leaves of some plant species, mostly belonging to the *Solanaceae* family (which includes vegetables such as potato, tomato and pepper) (Japelt & Jakobsen, 2013). Wide variations have been reported in how much vitamin D_3 and 7-DHC they contain[11]. It is not yet known if the edible portions also contain vitamin D_3.

2.16 Food sources of vitamin D_2 are limited. Wild mushrooms are a rich natural source, containing approximately 13-30 µg (520-1200 IU) per 100g fresh weight (Mattila et al., 1994). Cultivated mushrooms do not contain high amounts of vitamin D_2 since they are grown in the dark but UVB treated vitamin D_2 enhanced mushrooms are now commercially available.

2.17 Foods are fortified with either vitamin D_3 or D_2. 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 2013[12]. However, most margarines and fat spreads are 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.

2.18 European Union (EU) law (Directive 2006/141/EC) stipulates vitamin D fortification of infant formula (1-2.5 µg/40-100 IU per 100 kcal) and follow-on formula (1-3 µg/40-120 IU per 100 kcal).

2.19 In the US, almost all milk[13] is fortified with vitamin D on a voluntary basis (9.6 µg/L; 385 IU/L) (FDA, 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 mandatory[14] (1-2.5 µg or 40-100 IU per 100 kcal).

2.20 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 mandatory[15] 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).

2.21 Dietary vitamin D supplements contain either vitamin D_2 or D_3. Vitamin D supplements can also be administered by intramuscular injection. The contribution that supplements make to vitamin D intakes in the UK is considered in chapter 8.

[11] Vitamin D_3 (< 0.1-0.28 µg/g dry weight; 0.1-42 µg/g fresh weight); 7-DHC (0.2-1.3 µg/g dry weight; 5-58 µg/g fresh weight).
[12] 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/).
[13] Assumed that this refers to dairy fluid milk and not plant based beverages.
[14] FDA. Code of Federal Register Title 21, Sec.107.100 infant formula: nutrient specifications (Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSerach.cfm
[15] Food and Drug Regulation. Available online at http://laws.justice.gc.ca/PDF/Regulation/C/C.R.C,_c._870.pdf

Metabolism

Absorption of dietary vitamin D

2.22 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 transported through the lymph into the systemic circulation (Dueland et al., 1983).

2.23 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 (Grossmann & Tangpricha, 2010); however, the authors noted the limited number of studies that have investigated this issue.

2.24 An RCT (Biancuzzo et al., 2010) that compared vitamin D (25 µg/1000 IU per day) absorption 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

2.25 Cutaneously produced vitamin D_3 enters the extracellular fluid before diffusing into dermal capillaries (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). Dietary vitamin D_2 and D_3 are transported in chylomicrons via the lymph and blood plasma to the liver.

2.26 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 vitamin D is about 4-6 hours (Mawer et al., 1971).

Conversion to active metabolite

2.27 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(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 $1,25(OH)_2D$.

2.28 The difference in the side chain between vitamin D_2 and D_3 is maintained during metabolism, i.e., vitamin D_3 is converted to $25(OH)D_3$ and then to $1,25(OH)_2D_3$; vitamin D_2 is converted to $25(OH)D_2$ and then to $1,25(OH)_2D_2$ (Jones et al., 1998).

2.29 Although vitamin D_2 undergoes similar metabolic transformations to vitamin D_3, it is unclear if all details of regulation and biological activity are identical to those of vitamin D_3 (Henry, 2011). Vitamin D_2 and its metabolites have a lower binding affinity to DBP than vitamin D_3 and its metabolites (Houghton & Vieth, 2006).

Hydroxylation to 25(OH)D

2.30 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 D_2 and D_3 with equal efficiency (Strushkevich et al., 2008). Other 25-hydroxylases have been found to have different activities for vitamin D_2 and vitamin D_3 (Bikle, 2009).

2.31 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)$_2$D

2.32 In the kidney, 25(OH)D is further hydroxylated to 1,25$(OH)_2$D, its biologically active form, or to 24,25 dihydroxyvitamin D [24,25$(OH)_2$D]. Production of 24,25$(OH)_2$D is usually the first step in the metabolic pathway to inactivate 25(OH)D, which prevents vitamin D intoxication (Norman, 2008). Plasma concentration of 24,25$(OH)_2$D is directly related to 25(OH)D concentration.

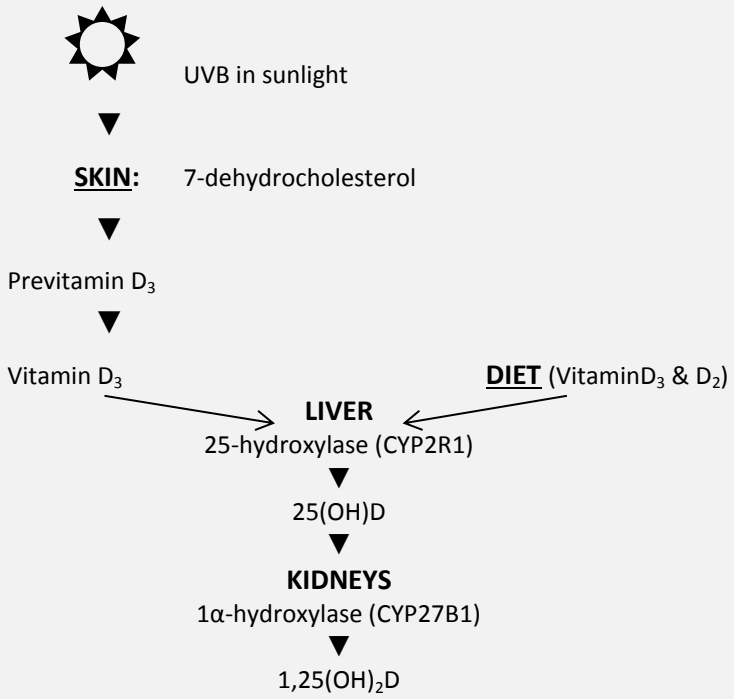
2.33 The conversion of 25(OH)D to 1,25$(OH)_2$D is catalysed by CYP27B1, a mitochondrial P450 enzyme with 1α-hydroxylase activity which is produced in the proximal renal tubule (Prentice et al., 2008; Bikle, 2009). Conversion to 24,25$(OH)_2$D is by the 24-hydroxylase enzyme, CYP24 (Norman, 2008).

2.34 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 plasma concentrations of 25(OH)D and 1,25$(OH)_2$D (Need et al., 2000; Lips, 2001; Vieth et al., 2003).

2.35 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 et al., 1998).

2.36 Renal production, which is the principal source of 1,25$(OH)_2$D in the plasma, mediates the functions of the vitamin D endocrine system. However, a number of other tissues also have the ability to produce 1,25$(OH)_2$D. 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$(OH)_2$D production does not generally increase 1,25$(OH)_2$D concentrations in the circulation (Norman, 2008) and its effects appear to be restricted to paracrine and autocrine functions within these tissues.

FIGURE 2: Vitamin D metabolism pathway



Physiological regulation

Regulation of 25(OH)D production

2.37 Plasma 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 plasma 25(OH)D is about 2-3 weeks (Lund et al., 1980) while the half-life of $1,25(OH)_2D$ is less than 4 hours (Holick, 2004a).

2.38 Plasma or serum 25(OH)D concentration is widely used as a biomarker of vitamin D status because of its long half-life in the circulation and because it is not subject to tight homeostatic control (DeLuca, 2008; Norman, 2008; Bikle, 2009). Biomarkers of vitamin D status are considered in chapter 4.

2.39 Plasma 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 plasma (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, rate of 25(OH)D uptake and the rate of conversion to other metabolites (such as $1,25(OH)_2D$, $24,25(OH)_2D$). Other factors affecting plasma 25(OH)D concentration include the volume of extracellular fluid and DBP concentration (Dueland et al., 1983; Orwoll & Meier, 1986; Lips, 2001; Gascon-Barre, 2005; Liang & Cooke, 2005; Bolland et al., 2007). Serum concentration of 25(OH)D has also been reported to decrease during the acute-phase response to inflammation (Silva & Furlanetto, 2015) (see paragraph 4.10).

2.40 In addition, different polymorphisms[16] of DBP have different affinities and transport efficiencies for 25(OH)D (Speeckaert et al., 2006). Evidence from children with calcium-responsive rickets (Pettifor,

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

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

2.41 Synthesis of $1,25(OH)_2D$ in the kidney is tightly regulated. Upregulation is through the action of parathyroid hormone (PTH) and down-regulation is through fibroblast growth factor 23 (FGF23) and direct negative feedback by $1,25(OH)_2D$ itself (Henry, 2011).

2.42 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 et al., 2008) which increases renal synthesis of $1,25(OH)_2D$ (Henry, 2011). $1,25(OH)_2D$ exerts a direct negative feedback by downregulating 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 that converts $1,25(OH)_2D$ and 25(OH)D to water-soluble compounds which are excreted through bile (Jones et al., 1998).

2.43 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 downregulates $1,25(OH)_2D$ 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 (Shimada et al., 2004; Segawa et al., 2007).

2.44 Extra-renal 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

2.45 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).

2.46 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).

2.47 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

2.48 Adipose tissue is considered to be the major storage/sequestration site for vitamin D (Rosenstreich et al., 1971; Mawer et al., 1972) although there is some evidence that muscle may also be a storage tissue for 25(OH)D (Girgis et al., 2014).

2.49 A number of studies have reported adiposity and body mass index (BMI) to be inversely related to serum 25(OH)D concentrations (Liel et al., 1988; Arunabh et al., 2003; Parikh et al., 2004; Snijder et al., 2005) 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 (Zittermann et al., 2009; Tzotzas et al., 2010).

2.50 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

2.51 $1,25(OH)_2D$ 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.

2.52 The VDR has a high affinity for $1,25(OH)_2D_3$ (Norman, 2008). $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ 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 et al., 1998). VDRs are also present in a wide range of other cells and tissues including macrophages, lymphocytes, skin keratinocytes, pancreatic ß-islet cells, ovarian tissue, mammary epithelium, neuronal tissue, lung, gonads, prostate, placenta, and adipose tissue (Jones et al., 1998; Norman, 2008). Its function in these tissues is not fully understood.

2.53 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 & Slatopolsky, 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

2.54 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 non-specified bases within the promoter region of target genes, resulting in activation or repression of transcription (Rachez & Freedman, 2000; Christakos et al., 2003; DeLuca, 2004).

Non-genomic response

2.55 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

2.56 In addition to behavioural and environmental factors, twin and family studies suggest a genetic component to the inter-individual variability in plasma 25(OH)D concentrations. Rates of heritability have been estimated to range from 29 to 80% (Hunter et al., 2001; Shea et al., 2009).

2.57 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.

2.58 A number of more common polymorphisms in genes encoding proteins involved in vitamin D metabolism have been identified. Two meta-analyses of genome-wide association studies[17] (Ahn et al., 2010; Wang et al., 2010b) examined associations of single nucleotide polymorphisms (SNPs[18]) in such genes with serum 25(OH)D concentrations. Ahn et al. (2010) included 9 cohorts from the USA and Finland (n=4501). 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/DHCR7[19] locus (rs3829251). Wang et al. (2010a) included 15 cohorts from the USA, Canada and Europe (n=16,125). 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.

2.59 These findings suggest that common polymorphisms in genes involved in vitamin D metabolism might influence serum/plasma 25(OH)D concentrations. The functional relevance of these findings is not clear.

Biological activity of vitamin D_2 versus vitamin D_3

2.60 Vitamin D_2 and vitamin D_3 both elevate plasma 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 plasma 25(OH)D concentrations (Lanham-New et al., 2010; Cashman, 2012; Logan et al., 2013; Swanson et al., 2014). Several biologically plausible mechanisms have been suggested that could contribute to the greater capacity of vitamin D_3 over D_2 to maintain higher 25(OH)D concentrations over time (reviewed in Houghton & Vieth, 2006).

2.61 Results from studies that have compared the effectiveness of D_2 and D_3 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 D_3 (p=0.006) but heterogeneity between the studies was high (I^2=81%). Separate meta-analyses, according to method of vitamin D administration, found a significantly larger increase in serum 25(OH)D concentration with vitamin D_3 compared with vitamin D_2 supplementation in 3 studies using single bolus doses (p=0.04) while there was no significant difference between vitamin D_2 and D_3 in 5 studies that used daily supplementation (p=0.06). Heterogeneity was much higher in the studies

[17] The entire human genome is searched to identify associations with the phenotype of interest.
[18] SNPs occur when a single nucleotide in the genome sequence is altered.
[19] NADSYN1 encodes nicotinamide adenine dinucleotide synthetase-1 which catalyses the final step in the biosynthesis of nicotinaminde 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.

using a bolus dose (I^2=77%*)* compared to those administering daily supplementation (I^2=44%). Conclusions regarding any differences in biological activity between vitamin D_2 and D_3 could not be drawn from this meta-analysis because of a number of limitations, including: small number and size of studies (n=19-89); variability in 25(OH)D assay methodology (see chapter 4); differences in dose size and frequency and in treatment and follow-up time. Additionally, the doses used in the studies were very high and effects may be different at lower doses.

2.62 Subsequent RCTs support the suggestion that vitamin D_3 is more effective than vitamin D_2 in raising serum 25(OH)D concentration. An RCT in New Zealand (Logan et al., 2013), conducted in winter, compared effects of 25 µg/d (1000 IU) of vitamin D_2, D_3 and placebo over 25 weeks on serum 25(OH)D concentration of adults (n=95; 18-50 y). After 25 weeks, serum 25(OH)D concentrations of participants in the placebo group were significantly lower than those in the vitamin D_2 and D_3 groups (both $p< 0.001$) and was significantly lower in in the vitamin D_2 supplemented group compared with the vitamin D_3 supplemented group ($p< 0.001$).

Toxicity

2.63 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 (EVM, 2003)[20].

2.64 Prolonged sunlight exposure does not lead to excess production of cutaneous vitamin D because endogenously produced previtamin D_3 and vitamin D_3 are photolysed to inert compounds (see paragraph 2.10). 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).

2.65 Animal studies suggest that plasma 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 D_2 or D_3 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 concentrations above 375-500 nmol/L (Vieth, 1990; Jones, 2008).

2.66 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.

2.67 Effects of high intakes of vitamin D, including thresholds for risk, single-dose acute toxicity vs sustained exposure, are considered further in chapter 7.

[20] Expert Group on Vitamins and Minerals.

Physiological role

Calcium & phosphate regulation

2.68 The major function of $1,25(OH)_2D$ 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).

2.69 Plasma 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 $1,25(OH)_2D$ which increases plasma calcium concentration by stimulating intestinal calcium absorption, renal calcium reabsorption and bone resorption. The subsequent increase in plasma calcium concentration is sensed by the parathyroid glands and PTH secretion is decreased (DeLuca, 2004).

2.70 In the intestine, $1,25(OH)_2D$ 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, $1,25(OH)_2D$ interacts with VDR in the osteoblast to increase expression of receptor activator of NFκB ligand (RANKL); this increases the production of osteoclasts which release calcium into the circulation (Christakos et al., 2003).

2.71 If plasma 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).

2.72 $1,25(OH)_2D$ 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).

Calcium & vitamin D interactions

2.73 Animal data have suggested that inadequate calcium intakes could cause changes in the physiological response to vitamin D (Berlin & Bjorkhem, 1987; Clements et al., 1987). Interactions between vitamin D and calcium may have implications for the regulation of plasma 25(OH)D concentrations and its catabolism and, as a consequence, the dietary vitamin D requirement.

2.74 Observational studies in humans have been inconsistent. Some have reported calcium intakes as a significant determinant of serum 25(OH)D concentration (van der Wielen et al., 1995; Andersen et al., 2005; Hill et al., 2006) while others have found no effect (Hill et al., 2008). RCTs that have investigated the influence of calcium intake on 25(OH)D concentration have also been inconsistent. One RCT (Berlin & Bjorkhem, 1988) in healthy men (n=28; mean age, 29y; mean calcium intake=800 mg/d) reported that calcium supplementation (2g/d) for 6-7 weeks significantly increased mean serum 25(OH)D concentration in the intervention group (from 73 to 94 nmol/L; $p< 0.05$) and was significantly different ($p<0.005$) from that of the control group (67 to 71 nmol/L) at the end of the study. In contrast, two other RCTs (Goussous et al., 2005; McCullough et al., 2009) reported no effect of additional calcium intakes on serum/plasma 25(OH)D in healthy adults.

2.75 Another RCT[21] (Cashman et al., 2014a) investigated the effect of habitual calcium intake on serum 25(OH)D concentration. Healthy adults (n=125; age, ≥ 50y; mean calcium intake=814 mg/d) were stratified according to habitual calcium intake (< 700 compared with > 1000 mg/d; mean intakes, 496 & 1437 mg/d for low and high calcium intake groups respectively) and received either vitamin D_3 (20 µg/800 IU per day) or placebo for 15 weeks throughout winter. Mean serum 25(OH)D concentration increased significantly ($p \leq 0.005$) in the vitamin D_3 group and decreased significantly in the placebo group ($p \leq 0.002$) and were of the same magnitude irrespective of calcium intake. These findings suggest that calcium intake does not modify the requirement for vitamin D within the range of calcium intakes studied. However, since this study was conducted in adults without metabolic bone disease, the findings may not be applicable to children (who have higher calcium requirements because of increased metabolism) or to those with metabolic bone disease.

Physiological requirements by life-stage

Infants

2.76 Vitamin D, together with calcium and phosphorus, is required during infancy and early childhood (< 3y) 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 a failure or delay of endochondral calcification at the growth plates of the long bones which results in rickets and an accumulation of excess unmineralised osteoid (bone matrix) in all bones; the low mineral to bone matrix ratio in bone results in osteomalacia (Pettifor, 2012). 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

2.77 Adolescence is a critical developmental period for bone health when there is rapid growth. Vitamin D is important for bone accretion during this time of skeletal development. Although rickets is most commonly observed during infancy and in young children, it can also occur during the pubertal growth spurt and adolescence. Children presenting with rickets have histological features of both rickets and osteomalacia. Once the growth plates of the long bones have fused during adolescence, only features of osteomalacia are found (Pettifor, 2012). 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

2.78 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; DH, 1998).

2.79 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 musculoskeletal and non-musculoskeletal health outcomes. Relationships between vitamin D status and health outcomes are considered in chapter 6.

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

Pregnancy and lactation

2.80 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 per day) are currently recommended during pregnancy to ensure that the mother and, therefore, the fetus are not deficient in vitamin D and to avoid neonatal hypovitaminosis (DH, 1991; DH, 1998). Breast milk is not considered to be a significant source of vitamin D or its metabolites. The reported vitamin D content of breast milk differs across studies because it varies with the type of milk measured (foremilk or hindmilk[22]) and the time of day it is collected. For some breast fed babies, vitamin D and 25(OH)D contained in breast milk could make a significant contribution to their vitamin D intake since dietary 25(OH)D has been reported to be 5-times more effective than vitamin D_3 in raising serum 25(OH)D concentration (Cashman et al., 2012) (see paragraph 5.4). It is also likely that vitamin D is well absorbed from breast milk as fat absorption is particularly efficient.

2.81 Associations between vitamin D status during pregnancy and lactation on health outcomes in the mother and baby are considered in chapter 6.

[22] At the start of the feed, the milk (foremilk) is relatively low in fat and quenches the baby's thirst. Later during the feed, the milk (hindmilk) becomes richer in fat, which provides calories for growth.

3. Photobiology of vitamin D

Ultraviolet radiation

3.1 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.

3.2 Solar UVR forms the part of the electromagnetic spectrum from wavelengths of about 100-400 nm. The International Commission on Illumination (CIE, 2011)[23] 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); and UVC (100–280 nm).

3.3 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.

3.4 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.

3.5 Ground-level UVR consists of two major components: 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.

3.6 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.

3.7 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, during winter and at 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

[23] From its French title: Commission Internationale de l'Eclairage.

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 percent of the global UVR exposure[24] is delivered during the four hours centred around noon.

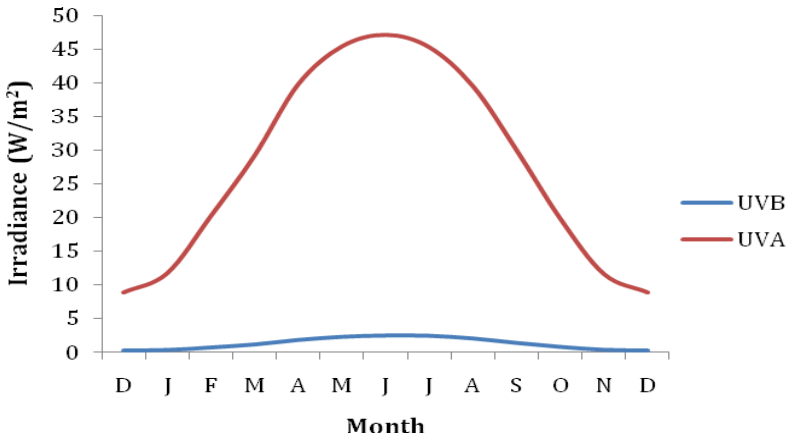
Biological effects of solar UVR exposure

3.8 Solar UVR has been associated with beneficial and harmful biological effects. Synthesis of vitamin D is the only established benefit of solar UV exposure. 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).

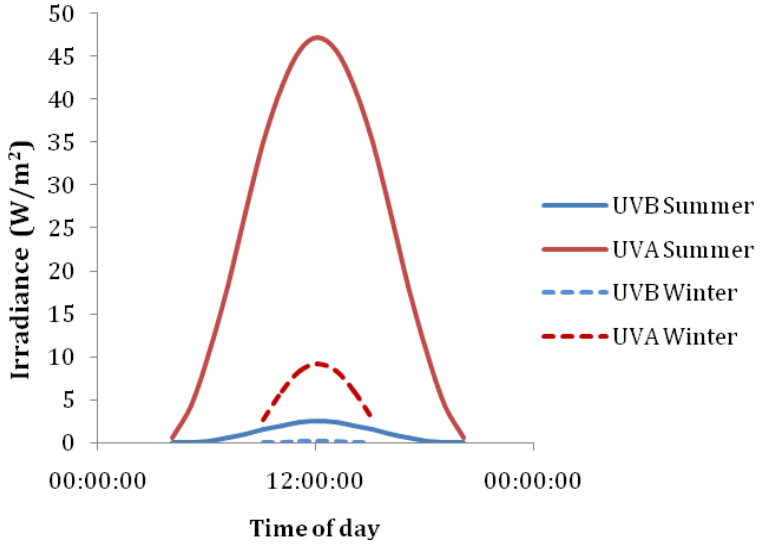
3.9 Adverse biological effects of UVR exposure include damage to the skin (erythema or sunburn, photoageing and skin cancer) and eyes (photokeratitis, cataract and age-related macular degeneration). The focus of public health advice is on sun avoidance and protection to reduce the risk of sunburn and skin cancer.

FIGURE 3 - Variation of UVB and UVA irradiance at Chilton, UK[25]

(a) Monthly at noon on 21st day of each month



(b) daily at summer and winter solstices



[24] The integrated total exposure dose of biologically weighted UVR falling on a horizontal surface.
[25] These data have been modelled taking total ozone into account.

Erythema and skin cancer

3.10 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; Fitzpatrick, 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).

3.11 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.

3.12 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.

3.13 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 Figures 3a and 3b with the CIE erythema action spectrum. Thus, the minority UVB physical component becomes the major biological component.

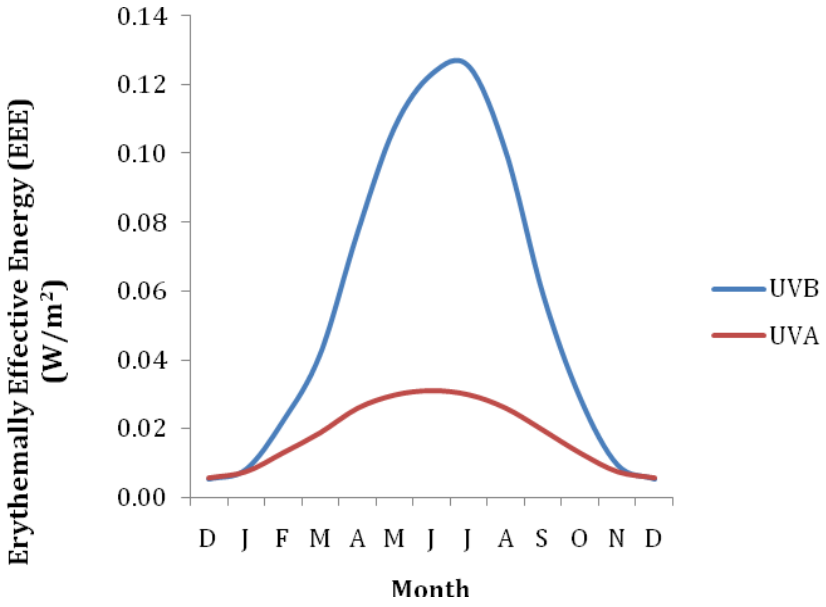
3.14 Skin cancer is initiated by UVR-induced damage to epidermal DNA. The most important photolesion is the cyclobutane pyrimidine dimer (CPD). The action spectrum for CPD formation is very similar to that for erythema and CPD is thought to be a trigger for erythema. The presence of erythema indicates 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.

3.15 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 cancer[26]) is likely to also reduce vitamin D synthesis.

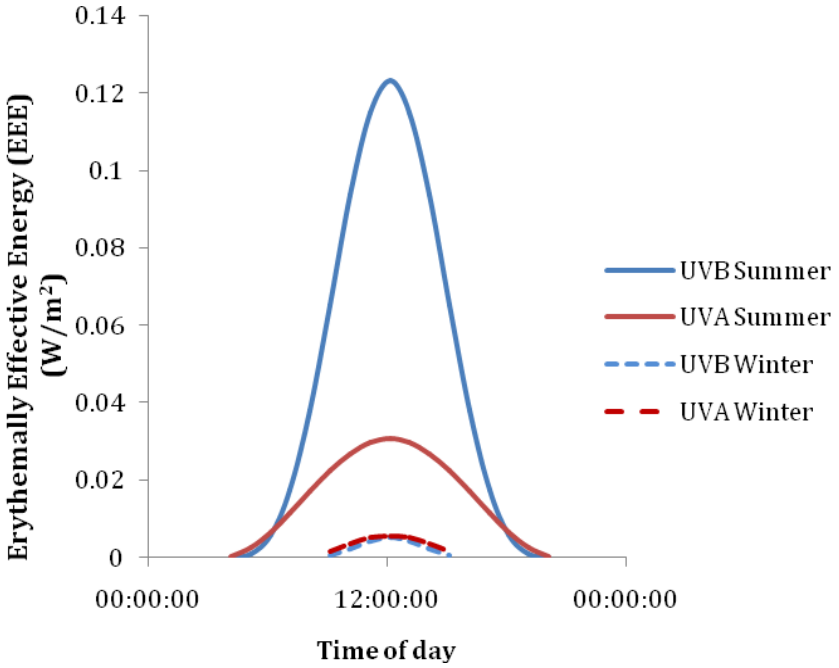
[26] Non melanoma skin cancer has an action spectrum that is similar to erythema.

FIGURE 4 - Variation of UVB and UVA erythemally effective energy at Chilton, UK[27]

(a) Monthly at noon on 21st day of each month



(b) Daily at summer & winter solstices

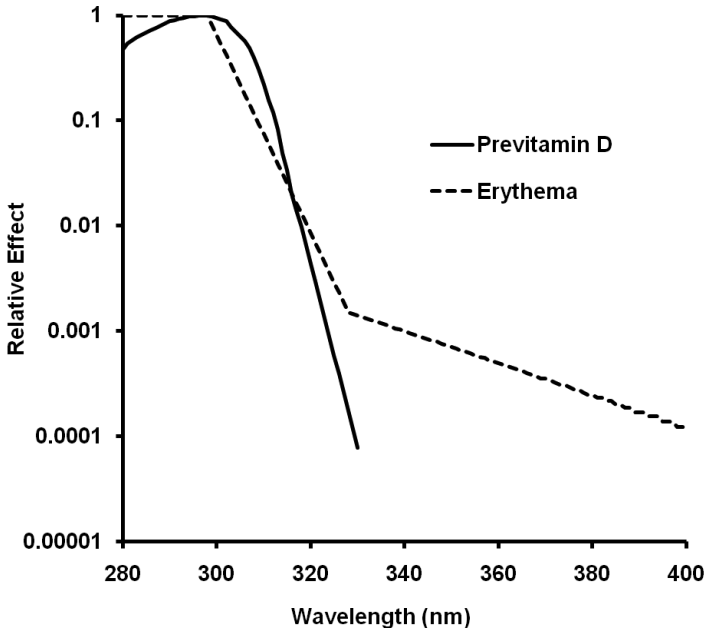


[27] Based on the data in Figure 3

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

3.16 The validity of the official CIE action spectrum for the photoconversion of 7-DHC 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 concluded 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.

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



3.17 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 skin synthesis of vitamin D

3.18 UVB radiation is sufficient for year round vitamin D synthesis at latitudes below ~37°N. At higher latitudes, vitamin D is not synthesised during the winter months (Webb et al., 1988). However, the extent of the effect of latitude on vitamin D synthesis in the UK is not clear. While it probably has some influence, it could be relatively small compared to other factors. A study which compared serum 25(OH)D concentrations in postmenopausal women (< 65y) in the North (Aberdeen; 57°N) and South (Guildford; 51°N) of the UK reported a difference of approximately 10 nmol/L (Macdonald et al., 2011). However, the difference in serum 25(OH)D concentration 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 spend less time outdoors and expose less skin.

Seasonal variation in serum 25(OH)D concentration

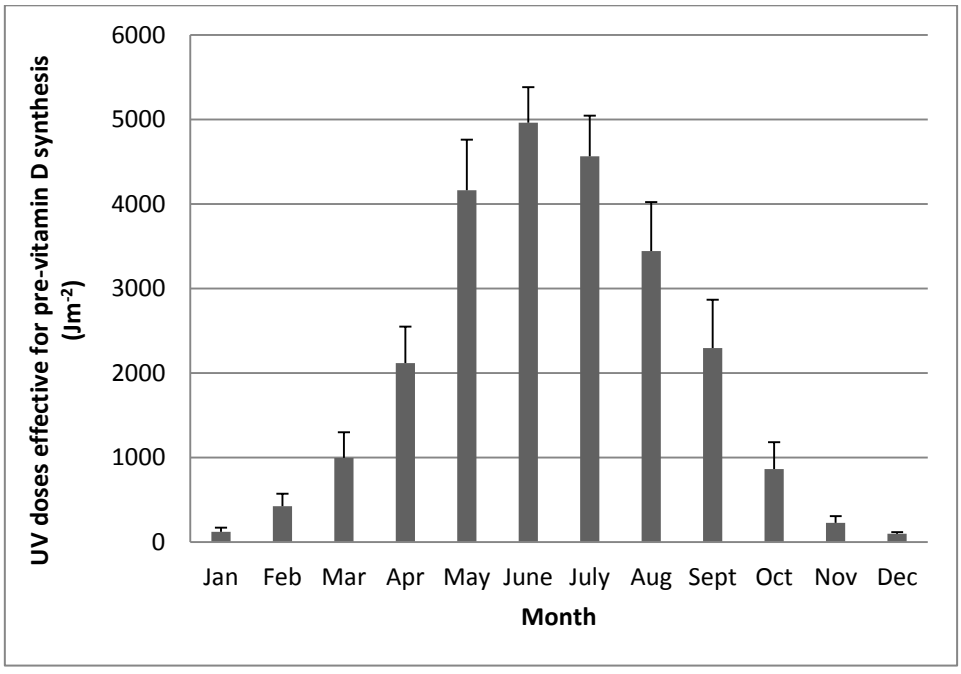
3.19 The main factor affecting skin synthesis is availability of UVB radiation (Webb & Engelsen, 2006). There is a well-observed seasonal cycle in serum/plasma 25(OH)D concentrations in the UK and other countries at mid-high latitudes (Poskitt et al., 1979; Beadle et al., 1980; Livesey et al., 2007) which relates to the greater UVB content of solar UVR in summer.

3.20 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 April. Maximum serum 25(OH)D concentrations are 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 mean concentration was highest in September (71 nmol/L) and lowest in February (46 nmol/L).

3.21 A seasonal difference in serum/plasma 25(OH)D concentration of the UK population has also been observed in national surveys (see chapter 8). Figure 6 shows the estimated monthly average UV doses (Jm^{-2}) effective for pre-vitamin D synthesis in the UK from 2008-2012.

3.22 The metabolic implications of seasonal variation in serum 25(OH)D concentration are currently unknown.

FIGURE 6: The modelled monthly averaged UV doses (Jm^{-2}) effective for pre-vitamin D synthesis in the UK from 2008-2012. *(Courtesy of O'Neill, Kazantzidis, Webb, Cashman, EU ODIN Project[28])*



[28] This work was based on original model development funded by UK Department of Health.

Effect of skin pigmentation on skin synthesis of vitamin D

3.23 Epidemiological studies consistently show that, under given climatic conditions, people with dark 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 differences in physiology or differences in lifestyles (e.g., sun avoidance behaviour). However, dark 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 concentrations of different ethnic groups.

3.24 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 a proportion of the incident UVB 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 dark 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 (Lo et al., 1986; Farrar et al., 2013).

3.25 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; Libon et al., 2013) compared serum 25(OH)D concentrations after a single total body UVB exposure in white (n=20; skin type II-III) and black (n=11; skin type VI) skinned individuals. Serum 25(OH)D concentrations increased significantly in the white-skinned ($p < 0.0001$, days 2 & 6), but not in the black-skinned ($p=0.843$, day 2; $p=0.375$, day 6) individuals.

3.26 Farrar et al. (2011) examined the effect of a controlled dose of UVR exposure (3 times/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 UVR 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 ($p< 0.0001$).

3.27 In contrast, (Bogh et al., 2010) found no significant correlations between constitutive or facultative skin pigmentation[29] and serum 25(OH)D concentration. In addition, the increase in serum 25(OH)D concentration after identical UVB exposure did not differ between light and dark skinned groups. 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 with shorter wavelengths (< 295 nm). These wavelengths penetrate the skin less well and pre-vitamin D synthesis may occur above the melanin layer (Bjorn, 2010). Other studies (Stamp, 1975; Brazerol et al., 1988; Matsuoka et al., 1990) have reported no differences by skin colour in serum 25(OH)D concentration response after UVB exposure. Brazerol et al. (1988) used a UVB source which emitted as much short wavelength radiation than that used by Bogh et al. (2010). The study by Stamp (1975) did not provide details of the UVB source.

[29] *Constitutive* skin pigmentation is the natural, genetically determined colour of the skin. *Facultative* skin pigmentation arises from exposure to UV light and other environmental factors.

Effect of ageing on skin synthesis of vitamin D

3.28 In cross-sectional studies, lower serum 25(OH)D concentrations have been observed in older compared to younger people (Baker 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 that this could explain lower serum 25(OH)D concentrations observed in older people. It is uncertain, however, whether the lower 7-DHC concentration is a limiting factor if there is ample exposure to sunlight.

3.29 It has also been suggested that the lower concentration 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 (70-88y) who were not very active (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/400 IU per day vitamin D supplements) and free-living older people (no supplements), reported that the supplements kept year round serum 25(OH)D concentrations > 37.5 nmol/L (Webb et al., 1990). This was similar to serum 25(OH)D concentrations of the free-living older people in spring and summer.

3.30 It is possible that the lower serum 25(OH)D concentrations reported in older people could also be due to the development of conditions that become more common with increasing age (such as reduced liver and/or kidney function). Although lower concentrations are more generally due to reduced exposure to sunlight rather than impaired metabolism of vitamin D, severe liver disease may impair hydroxylation of vitamin D to 25(OH)D.

3.31 In the UK National Diet and Nutrition Survey (2008/9-2011/12), mean plasma 25(OH)D concentrations were not found to be lower in adults aged over 65y compared to those aged 19-64y; there was also no difference in the proportion with plasma 25(OH)D concentration < 25 nmol/L.

Effect of sunscreen on skin synthesis of vitamin D

3.32 Sunscreen use is recommended for the prevention of sunburn and skin cancer, which has raised concerns that its application may inhibit or prevent vitamin D synthesis. It is not possible to draw definitive conclusions from published studies (Springbett et al., 2010). A review of studies which examined whether chronic sunscreen use reduced vitamin D production (Norval & Wulf, 2009) reported that the majority of people did not use sunscreen at the recommended concentration and did not apply it to all exposed areas of skin. It concluded that although sunscreens can significantly reduce the production of vitamin D under very strictly controlled conditions, their normal usage does not generally prevent vitamin D synthesis. Overall, the data suggest that vitamin D synthesis is still possible even 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

3.33 Few studies have compared the effect of UVR with that of 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) (3 times/week) for 6 weeks with a daily dose of vitamin D_3 (40 µg/1600 IU) in participants (n=73) with serum 25(OH)D concentration ≤ 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 D_3 supplemented group (from 23·3 to 60·6 nmol/L) (p=0.02).

3.34 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 either narrow band solar radiation exposures (3 times/week) or vitamin D_3 supplements (20 μg/800 IU per day). Mean baseline serum 25(OH)D concentration of participants was 53 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

3.35 NHS Choices advice on safe sun exposure[30] follows that from Cancer Research UK's Sunsmart[31] campaign:

- **S**pend time in the shade between 11.00 am and 3.00 pm.
- **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.

3.36 The British Photodermatological Group (British Association of Dermatology) provides similar advice to the above (i.e., avoid sunlight exposure between about 11.00 am and 3.00 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 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 as a reason to stay outside longer or to avoid more reliable protective measures such as clothing and shade.

3.37 The WHO's INTERSUN programme on safe sun exposure advises that shade, clothing and hats provide the best protection against the sun and that application of sunscreen is necessary to those parts of the body that remain exposed (e.g., face and hands). It makes the following recommendations: limit the time spent in the sun between 10.00 am and 4.00 pm; use the UV index to plan outdoor activities in ways that prevent overexposure to the sun's rays and take special care to adopt sun safety practices when the UV Index predicts exposure levels of moderate or above; seek shade when UV rays are the most intense; wear protective clothing (hat with wide brim, sunglasses, tightly woven loose-fitting clothes); apply a broad-spectrum sunscreen of SPF 15+ liberally and reapply every two hours, or after working, swimming, playing or exercising outdoors.

3.38 The National Institute for Health and Care Excellence (NICE) has published guidance on the risks and benefits of sunlight exposure (NICE, 2016[32]). It advises that most people can make sufficient vitamin D by going out for short periods and leaving only areas of skin that are often exposed uncovered (such as forearms, hands or lower legs). People with dark skin (skin types V & VI) are advised that they may

[30] http://www.nhs.uk/Livewell/skin/Pages/Sunsafe.aspx
[31] http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/sun-uv-and-cancer/ways-to-enjoy-the-sun-safely
[32] https://www.nice.org.uk/guidance/ng34

need more sunlight exposure to produce the same amount of vitamin D as people with lighter skin generally but could be exposed for a longer time before risking sunburn and skin cancer. The advice for people with naturally very light skin or fair/red hair or freckles (skin types I & II) is that they do not need much time in the sun (less than the time it takes them to burn) to produce vitamin D but that they are at greater risk of sunburn and skin cancer than people with darker skins. Recommendations to protect skin from strong sunlight include covering up with suitable clothing, seeking shade and applying sunscreen (SPF 15+). It advises that sunscreen is not an alternative to covering up with suitable clothing/shade, but offers additional protection only if used liberally, carefully and repeatedly on all exposed skin (including straight after being in water, after towel drying or after sweating). It advises that infants aged under 6 months should be kept out of direct strong sunlight and children and young people need their skin protecting between March and October in the UK by covering up with suitable clothing, spending time in the shade (particularly between 11 am-3.00 pm) and wearing sunscreen.

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

Biochemical markers of vitamin D exposure

4.1 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.

4.2 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, which 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 (Cashman & Kiely, 2011; IOM, 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.

4.3 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 is the relationship between exposure and serum 25(OH)D concentration.

4.4 The appearance in 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 D_3 to 25(OH)D concentration at typical inputs of vitamin D_3 (whether cutaneous or oral). They also suggested 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 1α-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 high performance liquid chromatography (HPLC) analysis, this is not routinely performed and not used clinically (Norman, 2008).

4.5 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, 2004b; 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 plasma 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 upregulation of the CYP27B1 enzyme; plasma 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 plasma $1,25(OH)_2D$, which is less than 4 hours.

4.6 There is consensus that serum or plasma 25(OH)D concentration should be used to assess vitamin D status because it reflects the contributions from both diet and cutaneous 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 D_2 or D_3 was used, differing analytical techniques, study duration (6 weeks to > 2y), or age group of participants (Seamans & Cashman, 2009).

4.7 Serum 25(OH)D concentration was used as an indicator of vitamin D status by the IOM (IOM, 2011) and the UK and EU authorities (Commission of the European Communities, 1993; DH, 1991; DH, 1998; German Nutrition Society, 2012; Health Council of the Netherlands, 2012; Nordic Council of Ministers, 2014) 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).

4.8 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 a status marker in the face of a significant systemic inflammatory insult (Reid et al., 2011). By day 2 post-operatively there was a large increase in C-reactive protein (CRP)[33] concentration 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 concentration 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, although other studies suggest that this might be a consequence of changes in DBP (Waldron et al., 2013).

4.9 Serum concentrations of other lipid-soluble vitamins (A, E, K and some carotenoids) decrease during the systemic inflammatory response. While changes in CRP 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 chronic inflammatory conditions. 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 disease with an inflammatory component and not the cause.

4.10 A systematic review of longitudinal studies (n=8) evaluated the association of acute phase response with serum 25(OH)D concentration during an inflammatory state (Silva & Furlanetto, 2015). Serum 25(OH)D concentration was measured before & after elective surgery in 4 studies, during the acute phase response following intravenous treatment with nitrogen-containing bisphosphonates in 1 study and soon after diagnosis and during the course of an acute illness in 3 studies. Serum CRP concentration was used as an inflammatory marker in most studies. Serum 25(OH)D concentration decreased after the inflammatory insult in 6 studies with no change observed in 2 studies. However,

[33] CRP is an acute phase protein produced by the liver; plasma/serum concentrations rise in response to inflammation.

the authors cautioned care in the interpretation of these findings because of some heterogeneity in the included studies (e.g., the acute inflammatory response was due to several causes and serum 25(OH)D concentration was measured at different time points).

4.11 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, institutionalisation, use of sunscreen as well as physiological state of the individual such as BMI, extracellular volume, 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; Cashman & Kiely, 2011; IOM, 2011)**.**

4.12 Choice of measurement methodology can also influence the absolute quantification of serum concentrations of 25(OH)D (see chapter 5). In addition there is ongoing debate regarding thresholds for serum 25(OH)D concentration that indicate vitamin D deficiency, inadequacy and sufficiency.

4.13 As plasma 25(OH)D concentration increases, plasma 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 (Holick et al., 2011; IOM, 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 nature of the 25(OH)D-PTH relationship, and concentrations of PTH which may have adverse effects on bone health (IOM, 2011). In addition, plasma 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.

4.14 The ratio of serum 24,25$(OH)_2$D to 25(OH)D concentration has also been suggested as an indicator of vitamin D deficiency (Wagner et al., 2011; Kaufmann et al., 2014; Cashman et al., 2015) however, more research is needed about the utility of this ratio over that of serum 25(OH)D concentration alone.

Assessment of vitamin D exposure

4.15 Assessment of sunshine exposure and habitual dietary intake of vitamin D can be useful additions to the biochemical assessment of serum 25(OH)D concentration but both have their limitations.

4.16 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).

4.17 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 D_2 and D_3) 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

4.18 Serum concentration of *total* 25(OH)D (i.e., comprising the sum of $25(OH)D_2$ and $25(OH)D_3$) 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 D_2 and vitamin D_3 is widespread; however, some immunoassays do not detect 100% of $25(OH)D_2$ (see below).

4.19 A C-3 epimer[34] 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 biological function. If the C-3 epimer of 25(OH)D is found to have biological activity, it may 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 the estimate of *total* 25(OH)D in many of the assay methods in current use because of the inability to separate it from 25(OH)D.

4.20 Serum $24,25(OH)_2D_3$ concentration can range from 2% to 20% of serum total 25(OH)D concentration (Bosworth et al., 2012) and has been shown to increase in direct proportion to that of serum $25(OH)D_3$ concentration (Kaufmann et al., 2014; Cashman et al., 2015). The impact of pre-analytical factors (e.g., serum versus plasma, fasting versus non-fasting state, or time of day) on 25(OH)D concentration is not well defined.

4.21 While a variety of methods are available to determine serum or plasma 25(OH)D concentration, 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 $25(OH)D_2$, $25(OH)D_3$, $24,25(OH)_2D_3$ or the C-3 epimer of 25(OH)D.

4.22 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 $25(OH)D_2$ and $25(OH)D_3$ concentrations (and in some cases, the C-3 epimer and $24,25(OH)_2D_3$) from serum samples.

4.23 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 $25(OH)D_2$ and $25(OH)D_3$ (Hollis & Napoli, 1985) while others underestimate the $25(OH)D_2$ component and therefore provide correction factors to compensate for high $25(OH)D_2$ content (IOM, 2011). Some manufacturers of the antibody-based assays report > 100% cross-reactivity of the antibody with

[34] Epimers have identical molecular structure but differ in stereochemical configuration.

$24,25(OH)_2D_3$ 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). An important consideration is 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.

4.24 LC-based assays which use a tandem mass spectrometer (LC-MS/MS) allow discrimination between $25(OH)D_2$ and $25(OH)D_3$ 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

4.25 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.

4.26 The Vitamin D External Quality Assurance Scheme (DEQAS[35]) 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 indicate 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).

4.27 The issue of international standardisation of serum 25(OH)D measurement is also being addressed 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 and a number of the national health surveys around the world (HHS, 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 25(OHD concentration

4.28 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 25(OH)D concentration used to define low vitamin D status, which has ranged between 12.5 and 120 nmol/L (Zittermann, 2003). This is principally because different functional endpoints/outcome indicators used have different serum 25(OHD) concentration

[35] Based at Charing Cross Hospital, London, UK.

thresholds, although there has also been disagreement over the appropriate threshold concentration for a specific functional endpoint(s).

4.29 In the UK, for example, a serum 25(OH)D concentration of 25 nmol/L is currently used as a threshold (cut-off) for defining the lower limit of adequacy (DH, 1998), based on evidence suggesting risk of rickets and osteomalacia is increased at concentrations below this level.

4.30 The IOM, using bone health as the basis for developing Dietary Reference Intakes (DRIs) for vitamin D, proposed a serum 25(OH)D concentration of 40 nmol/L as the 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 concluded 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.

4.31 In contrast, the Endocrine Society Task Force on Vitamin D (Holick et al., 2011) concluded 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'.*

4.32 While both the IOM DRI committee and the Endocrine Society Task Force appeared to agree that there was insufficient evidence of a causative link between 25(OH)D concentration and any non-skeletal disease outcomes, others have proposed serum 25(OH)D thresholds between 50-120 nmol/L to reduce the risk of adverse non-skeletal outcomes (Zittermann, 2003; Holick, 2004b).

4.33 The wide variation in measurements of serum 25(OH)D concentration, 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

5.1 Humans have two routes of exposure to vitamin D:

i. Vitamin D_3 derived from synthesis in human skin on exposure to UVB containing sunlight.

ii. Dietary exposure through consumption of vitamin D_2 and D_3 in the form of naturally occurring foods, fortified foods and dietary supplements. Some animal derived foods may contain small amounts of 25(OH)D_3 in addition to vitamin D_3.

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

5.2 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)D_2 and 25(OH)D_3) to altered intake of vitamin D_2 and/or D_3 (plus 25(OH)D_3 in some cases). There are a number of considerations which may impact on this relationship.

5.3 Vitamin D_2 and D_3 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 vitamers are equally effective in raising and maintaining serum total 25(OH)D concentration (see paragraphs 2.60-2.62).

5.4 Data indicate that per μg of vitamin D compound consumed, 25(OH)D_3 (a minor dietary form) is approximately 5-times as effective as vitamin D_3 in elevating serum 25(OH)D_3 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). It may also be of relevance to the vitamin D content of breast milk.

5.5 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, 28y), Barger-Lux et al. (1998) reported that the larger the BMI, the smaller the rise in serum 25(OH)D concentration for any given dose of vitamin D. Forsythe et al. (2012), using data from RCTs (Cashman et al., 2008; Cashman et al., 2009), reported that BMI was negatively associated with change in serum 25(OH)D concentration following supplementation in older (n=109; age, ≥ 64y) but not younger (n=118; age, 20-40y) adults. 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).

5.6 Despite these considerations, the relationship between vitamin D (not distinguishing between vitamin D_2 and D_3) intake and serum 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/40 IU 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 D_3 over an extended winter period (Heaney et al., 2003).

5.7 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/2000 IU), 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 D_3 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 flattened off at a dose of 35 μg/d (1400 IU/d).

5.8 The IOM also reported a steeper rise in serum 25(OH)D concentration at vitamin D doses < 25 μg/d (1000 IU/d) and a slower more flattened response at doses ≥ 25 μg/d (1000 IU/d) 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/d). The 95th percentile of total vitamin D intake in national nutrition surveys in Europe is generally less than 15 μg/d (600 IU/d) (Flynn et al., 2009) so the intake range for many populations is likely to lie where the intake-status relationship is more linear.

5.9 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., 2011a). 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/d) (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 (40 IU) vitamin D (Cashman et al., 2008; Cashman et al., 2009; Viljakainen et al., 2009; Cashman et al., 2011b); 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 5.6).

5.10 The meta-regression analyses by Cranney et al. (2007) and Seamans & 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 skin synthesis of vitamin D is minimised. At times of the year when UVB sunlight is sufficient for skin 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 RCTs conducted at

latitudes > 49.5°N or S (which were used for derivation of the current US Recommended Dietary Allowance [RDA[36]] values) yielded quite different regression equations (resulting in lower RDA estimates). This highlights 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 during pregnancy and lactation

5.11 Serum 25(OH)D concentration remains stable during pregnancy (Kovacs, 2008) suggesting that the increase in serum 25(OH)D concentration in response to vitamin D supplementation of pregnant and lactating women is similar to that of non-pregnant or non-lactating women.

5.12 A Cochrane systematic review (De-Regil et al., 2016) reported that data from 7 intervention trials (n=868) showed that women receiving vitamin D supplements during pregnancy had higher 25(OH)D concentrations at term compared to those who received no intervention/placebo but the response was highly heterogeneous (I^2 = 99%), ranging from 16.3 (95% CI, 13.6-19.0) nmol/L (Mallet et al., 1986) to 152 (95% CI, 127-177) nmol/L (Brooke et al., 1980). The large effect size in the Brooke et al. (1980) study, which made a significant contribution to the observed heterogeneity, is hard to explain. Differences in vitamin D doses and regimens (5-50 µg/200-2000 IU per day, 875 µg/35,000 IU per week and single doses from 5000 µg/200,000 IU to 15,000 µg/600,000 IU) and differences in methods used to assess serum 25(OH)D concentration may also have contributed to the heterogeneity. Subgroup analysis suggested that women who received vitamin D supplements on a daily basis had a higher 25(OH)D concentration at term compared with women who received a single dose. The review did not report mean baseline serum 25(OH)D concentrations of the women in the included studies.

5.13 A systematic review (Thiele et al., 2013) identified 3 RCTs (Hollis & Wagner, 2004; Wagner et al., 2006; Saadi et al., 2009) that had evaluated the effects of maternal vitamin D supplementation (10-160 µg/400-6400 IU per day) during lactation on serum 25(OH)D concentration of exclusively breast fed infants. Maternal serum 25(OH)D concentration and the vitamin D content of breast milk increased significantly with vitamin D doses of 50-160 µg/d (2000-6400 IU/d) but not with doses of 10 µg/d (400 IU/d).

5.14 A cohort study in Denmark (við Streym et al., 2016) collected blood and breast milk samples from mothers (median age, 30.4 y) at 2 weeks (n=107), 4 months (n=90) and 9 months (n=48) postpartum and blood samples from infants at 4 and 9 months of age. Mean maternal postpartum plasma 25(OH)D concentration was 73.2, 64.9 and 50.7 nmol/L at 2 wks, 4 months and 9 months respectively. Concentrations of vitamin D and 25(OH)D were higher in hindmilk than in foremilk and correlated with maternal plasma 25(OH)D concentrations: median (IQR) concentrations in foremilk and hindmilk were 1.35% (1.04-1.84%) and 2.10% (1.63-2.65%) respectively of maternal plasma 25(OH)D concentration ($p<0.01$). Daily median (IQR) infant intakes of vitamin D and 25(OH)D from breastmilk were 0.10 µg/4 IU (0.02-0.40 µg/0.8-16 IU) and 0.34 µg/13.6 IU (0.24-0.47 µg/9.6-18.8 IU) respectively. Concentrations of vitamin D and 25(OH)D in breastmilk also showed a significant seasonal variation ($p<0.01$). Findings from this study suggest that daily supply of vitamin D from breast milk of healthy women, with plasma 25(OH)D concentrations > 50 nmol/L, is low and inadequate to meet infant

[36] 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.

dietary vitamin D requirements.

5.15 Most observational studies which have examined serum 25(OH)D concentration of breast fed infants are not relevant to the UK. However, a cross-sectional study in New Zealand (Wall et al., 2013) found 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; IQR[37], 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. However, serum 25(OH)D concentrations were higher in spring than in 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.

Relationship between vitamin D intake and serum 25(OH)D concentration by ethnicity

5.16 Data on dose-response effects of vitamin D intake on serum 25(OH)D concentration of individuals from ethnic groups in the UK are lacking.

5.17 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., 2014). Based on their findings from a 4-arm RCT (placebo, 25 µg/1000 IU, 50 µg/2000 IU, or 100 µg/4000 IU vitamin D_3 daily for 3 months), Ng et al. (2014) estimated that 41 µg/d (1640 IU/d) of vitamin D was required to maintain winter plasma 25(OH)D concentrations > 50 nmol/L in 97.5% of African American men and women (n=292; age, 30-80y). This is almost twice the amount established by the IOM (15-20 µg/600-800 IU per day) 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.

5.18 In contrast, Gallagher et al. (2013) reported that the increase in serum 25(OH)D concentration after daily vitamin D_3 supplementation (placebo, 10 µg/400 IU, 20 µg/800 IU, 40 µg/1600 IU, 60 µg/2400 IU, 80 µg/3200 IU, 100 µg/4000 IU, or 120 µg/4800 IU for 12 months) in older African American women (n=110; mean age, 67y) was similar to that observed in white women (n=163; mean age, 67y) in a similarly designed RCT (Gallagher et al., 2012) and that 20 µg (800 IU) per day of vitamin D was required to maintain 25(OH)D concentration > 50 nmol/L in 97.5% of African American and white women.

5.19 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; mean age 36.7y) and who were assigned to receive placebo or vitamin D_3 (10 µg/400 IU, 20 µg/800 IU, 40 µg/1600 IU or 60 µg/2400 IU) daily for 12 months, reported that mean baseline serum 25(OH)D concentration was lower in African American women (29 nmol/L) than the white women (36.4 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 (400 IU/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 (800 and 1600 IU/d) of vitamin D was required to

[37] Inter-quartile range.

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 concentration

5.20 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).

5.21 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 concentration (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 and rapidly taken up by the liver.

5.22 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/cm^2. 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 differences in the interventions (length and area of exposure; dose) and lack of complete data (Cranney et al., 2007).

5.23 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.

5.24 Laboratory studies (Bogh et al., 2010) report 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.

5.25 In a RCT which examined interactions between exposure dose[38] 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 and increasing the exposure dose from 0.75 to 3.0 SED decreased the effect of increasing body surface area. These data indicate 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.

5.26 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^oN) to half the MED (from artificial UVB; individual doses

[38] 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.

adjusted according to skin sensitivity) 3 times/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/d) of vitamin D_3 over the same period increased serum 25(OH)D concentration by 37 nmol/L (median, 60 nmol/L at end of trial).

5.27 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. Holick & Jenkins (2003) have 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.

5.28 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 UVR in the UK, % skin exposure). The results from this model indicate 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.

5.29 A UK group (University of Manchester) has examined and reported the efficacy of a simulated summer sunlight exposures in raising serum 25(OH)D concentration in UK white-skinned adults (Rhodes et al., 2010) and in adults of South Asian ethnicity (Farrar et al., 2011; Farrar et al., 2013). Studies were performed in winter (latitude 53.5°N) to avoid confounding by UVR. Participants wore clothing that revealed about 1/3 body surface area at commonly sun-exposed skin sites. In white skinned adults (n=109) low dose, sub-erythemal UVR (1.3 SED, 3 times/week for 6 weeks; total 23.4 SED/week) produced a mean final serum 25(OH)D concentration of 70 nmol/L (Rhodes et al., 2010). The UVR dose equates to about 15 minutes (ranging from 13 minutes if lying down to 17 minutes if standing) x6 weekly, exposing about 1/3 skin surface area, in unshaded midday summer sunlight. This estimate takes account of the fact that in real life, dorsal and ventral body surfaces are not exposed simultaneously to sunlight, and people adopt postures ranging from horizontal to the vertical randomly orientated to the sun (Webb et al., 2011).

5.30 Longitudinal studies at mid-UK latitude (Greater Manchester) have examined the relationship between dose of sunlight UVR exposure received throughout the year and serum 25(OH)D concentration in adults. In a longitudinal study of white skinned adults (n=109; age, 20-60y), personal UV dosimeter badges showed that participants were exposed to ~2% of ambient UVR (Webb et al., 2010), with median exposures of 3.7 SED/week in spring/summer and 0.1 SED in winter. Monthly serum 25(OH)D measurements revealed a seasonal pattern reaching a peak (mean 71 nmol/L) in September and a trough (45.8 nmol/L) in February. Sun exposure diaries indicated that relatively short, frequent solar exposures increased serum 25(OH)D concentration with participants spending a mean daily time outdoors in spring/summer of 9 (± 13) minutes/day on weekdays and 18 (± 23) minutes/day at weekends during peak ambient UVB times (11-00 to 13-00). Another longitudinal study (Kift et al., 2013) examined year-round serum 25(OH)D concentration and sunlight exposure in adults of South Asian ethnicity (n=125; age, 20-60y). Median serum 25(OH)D concentration was

22.5 nmol/L (IQR, 16.8-34.3) in summer and 14.5 nmol/L (IQR, 10-20.3) in winter. This was about 1/3 that of white skinned adults at the same latitude: 65.4 nmol/L (IQR, 49.7–78.6) in summer and 47.2 nmol/L (IQR, 29.0–59.2) in winter. The South Asian adults also had lower UV exposure recorded by their personal dosimeter badges (~1% ambient UV) and had exposed a smaller skin surface area.

6. Vitamin D and health outcomes

6.1 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 DRVs for vitamin D. The health outcomes examined were those considered to be of public health concern.

6.2 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 the observational evidence was largely confined to studies that compared health outcomes against serum 25(OH)D concentration since this reflects total exposure to vitamin D from both sunlight and diet (from natural sources, fortified foods and supplements). Observational studies which only examined the relationship between vitamin D intake and health outcomes were not considered.

6.3 Studies considered were those that examined whether vitamin D reduced the risk or incidence of specific health outcomes in the general healthy population rather than its effect as a therapeutic agent in pre-existing disease; i.e., disease prevention rather than cure.

6.4 The IOM report on *Dietary Reference Intakes for Calcium and Vitamin D* (2011) provided a comprehensive reference resource for consideration of the evidence. 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). The process for reviewing the evidence is described in more detail in chapter 1 (see paragraphs 1.14-1.21).

6.5 For each of the potential health outcomes considered, the first step was to make a judgement on whether the evidence suggested a relationship with vitamin D supplementation or serum 25(OH)D concentration. If data were lacking or inconsistent for a specific health outcome, then it 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 to assess whether a range 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 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 varied across studies and were predefined according to different criteria for deficiency. As a consequence, the selected cut-offs were very insecure and made it difficult to assess if there was a dose response relationship.

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

6.6 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 pigmentation, clothing, time of exposure, sun screen use, urban environment (can reduce/block sunlight), air pollution and cloud cover.

6.7 Although serum 25(OH)D concentration is a marker of exposure to vitamin D (from sunlight and the diet), various factors complicate its use in studies of the relationship between vitamin D and health outcomes. Since serum 25(OH)D concentration reflects exposure to vitamin D, its concentration will be affected by factors that influence skin synthesis of vitamin D (see previous paragraph). Another important consideration in observational studies is that people with a higher serum 25(OH)D concentration tend to be healthier than those with lower concentrations. This could be due to greater exposure to sunlight as a result of more outdoor physical activity and/or a healthier diet and/or prophylactic use of supplements.

6.8 In addition to the variability which affects serum 25(OH)D concentration (e.g., time of day/time of year blood sample taken), it 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. A more detailed consideration of problems relating to measurement of serum 25(OH)D concentration is provided in chapter 4.

6.9 Serum 25(OH)D concentration is also influenced by genetic variation and by physiological state; for example, concentrations are 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. 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). A further 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.

6.10 All these issues 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

6.11 Assessment of the evidence is divided into musculoskeletal (rickets, osteomalacia, bone health indices, fracture prevention, risk of falls and muscle health) and non-musculoskeletal (pregnancy and lactation, cancers, CVD & hypertension, all-cause mortality, immune modulation, infectious diseases, neuropsychological functioning, oral health and age-related macular degeneration) health outcomes. Consideration of each health outcome includes a short summary of the IOM findings for that outcome.

Musculoskeletal health outcomes (Tables 1-30, Annex 2)

Bone structure and metabolism

6.12 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. It 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).

6.13 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.

6.14 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 and constitute over 90% of adult bone cells, 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.

6.15 Bone mass accrual is rapid in the fetus and infant. 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.

6.16 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 genotype; however, hormones (calcium regulating hormones and sex hormones) and 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

6.17 Insufficient vitamin D during growth leads to the development of rickets (vitamin D deficiency rickets). If diagnosed early, vitamin D supplementation can reverse the skeletal changes 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 has been the threshold adopted to define increased risk of rickets (DH, 1998). Other causes of rickets include inadequate

calcium intake, although this is more common in developing countries, and in children with inadequate phosphorus intake.

6.18 Osteomalacia, like rickets, develops as a result of vitamin D deficiency. It commonly presents in adults as 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. It can occur in children with rickets and there are also reports of adolescents presenting with symptoms of osteomalacia (Ladhani et al., 2004; Ward et al., 2005; Das et al., 2006). 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, which can interfere with vitamin D metabolism.

6.19 Osteoporosis is a progressive skeletal disorder generally associated with ageing. It is characterised by reduced bone strength due to loss of bone mass and deterioration in the micro-architecture of trabecular bone which increases bone fragility and, as a consequence, risk of fracture (WHO, 1994). Fractures are most common at sites where trabecular bone predominates; i.e., at the spine, wrist and hips. It can affect both sexes, but women are at greater risk mainly due to the decrease in oestrogen production after the menopause, which accelerates bone loss to a variable degree. Factors which affect bone mass will influence the risk of developing osteoporosis (see paragraphs 6.15-6.16).

Assessment of bone health

6.20 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 computed 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.

6.21 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 (Ammann & 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 et al., 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. 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). Bone mineral measurements are more useful in prospective studies where changes are assessed over time.

6.22 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. The International Society for Clinical Densitometry (ISCD) has published guidelines for clinical assessment of bone in children (Bishop et al., 2014; Crabtree et al., 2014).

6.23 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

6.24 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 (Tables 1-30, Annex 2).

6.25 Evidence on rickets and osteomalacia was not considered by life stage but separately, prior to consideration of other musculoskeletal health outcomes. Data on musculoskeletal health outcomes other than rickets and osteomalacia were considered by life stage (pregnancy & lactation, infants up to 12m, children 1-3y, children 4-8y & adolescents 9-18y, adults < 50y and adults ≥ 50y) 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 function and stress fracture risk were considered in adults < 50y[39]; fracture prevention, risk of falls and muscle strength and function were considered in adults ≥ 50y.

Rickets

6.26 IOM Report: The IOM concluded that, overall, there was fair evidence for an association between low serum 25(OH)D concentration and confirmed rickets but the evidence for a threshold serum 25(OH)D concentration above which rickets did 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) but it was noted that many were from developing countries where calcium intake is low and could, therefore, be confounded by dietary calcium.

6.27 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).

6.28 The IOM concluded that if calcium intake was adequate, the risk of rickets was increased at serum 25(OH)D concentration < 30 nmol/L.

[39] Ages ranged from 16 to 35 years in the studies considered.

Evidence considered (Tables 1-5, Annex 2)

6.29 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).

6.30 A total of 44 studies were identified which included measurements of serum 25(OH)D concentration in children with rickets.

6.31 Individual baseline serum 25(OH)D concentration in the case reports (n=17) 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=9), 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). 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.

6.32 Rickets with unknown aetiology, often with serum 25(OH)D concentration < 25 nmol/L, is usually defined as vitamin D deficiency rickets. However, as many of the studies on rickets were from developing countries, the findings could be confounded by low calcium intakes. Since only 5 studies reported calcium intakes, it was 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 is the assumption of a correlation between serum 25(OH)D concentration in children with rickets and their skeletal abnormalities, since the time course from the onset of the disease is not known. Serum 25(OH)D concentration in all studies was measured in children who presented with features of rickets; none obtained measurements before development of rickets. In case reports, serum 25(OH)D concentrations of children with rickets may therefore be lower than normal because presentation at a hospital usually occurs at a more advanced stage of the disease.

6.33 A wider range of serum 25(OH)D concentrations is found in population studies. For example, a study in Australia (Munns et al., 2012) of children (n=398; age, 0.2-15y) 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 71% of children with wrist x-rays (n=95) had signs of radiological rickets; however, the difference in median serum 25(OH)D concentration between cases with radiological rickets (median, 18 nmol/L; range 5-45 nmol/L) and those without (20 nmol/L; range 8-45 nmol/L) was not statistically significant.

6.34 Based on the overall evidence, it is not possible to discern a clear threshold serum 25(OH)D concentration below which rickets occurs. However, individual serum 25(OH)D concentrations were below 25 nmol/L (the current threshold for defining the lower limit of adequacy[40]) in the majority of case reports and mean serum concentrations were < 25 nmol/L in the majority of other study types considered.

6.35 Although the risk of rickets is increased at serum 25(OH)D concentrations < 25 nmol/L, it is important to recognise that a serum concentration of 25 nmol/L is not a clinical threshold diagnostic of the

[40] DH, 1998.

disease and that most children in the general population who have a serum 25(OH)D concentration < 25 nmol/L will not develop rickets.

6.36 Studies describing cases of rickets in countries with predominantly white-skinned populations (e.g., UK, USA, Australia) mainly comprised infants from ethnic groups with dark skin colour. These infants represent a minority of births in those populations, suggesting a larger relative risk of rickets associated with infants and children from ethnic groups with dark skin colour. However, it is not clear if this difference in risk is due to skin colour *per se* or lifestyle differences since dark skin is one of a number of factors (including behavioural, cultural and biological) that could affect rickets risk and serum 25(OH)D concentrations of these population groups (see paragraph 3.23).

Summary - Rickets

6.37 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 by low calcium intake.

6.38 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 of children with rickets were < 25 nmol/L in the majority of studies examined.

6.39 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 and none provided measures of the serum 25(OH)D concentration prior to the onset of disease.

Osteomalacia

6.40 IOM Report: 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 by 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 6.45-6.46).

Evidence considered (Tables 6-7, Annex 2)

6.41 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

6.42 Gifre et al. (2011) examined the clinical manifestations and most frequent causes of osteomalacia in a group of patients in Spain (n=28; mean age, 55y) 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 criteria[41]. Mean serum 25(OH)D concentration was 15 nmol/L in patients with vitamin D osteomalacia.

[41] Defined as two of the following: low calcium, low P, elevated total alkaline phosphatase, radiographic findings.

6.43 Preece et al. (1975) measured serum 25(OH)D concentration in 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%.

Case reports

6.44 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 relate to patients of South Asian ethnic origin living in the UK.

Bone biopsy study (Priemel et al., 2010)

6.45 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 in Germany (n=401 males, mean age 58.7y; n=274 females, mean age 68.3y) to assess the minimum 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.

6.46 A critique of this study (Aspray & Francis, 2013) has drawn attention to a number of concerns, 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; the validity of post-mortem serum 25(OH)D measurement and the generalisability of the study (since no demographic data other than age and sex were presented). Other limitations of the study include: data were reported as scatter plots without further statistical analysis or adjustment for age (range, 20-100y) 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); and information on calcium intakes, which might also affect bone mineralisation, was not available.

Summery - Osteomalacia

6.47 Evidence on osteomalacia is limited mainly to case reports in which serum 25(OH)D concentrations ranged between 4 and 20 nmol/L.

6.48 Out of 2 cross-sectional studies of patients with osteomalacia, individual serum 25(OH)D concentrations were < 7.5 nmol/L in one study and mean serum 25(OH)D concentration was 15 (±5) nmol/L in in the other.

<u>Other musculoskeletal health outcomes (beyond rickets and osteomalacia) by life stage</u>

<u>Pregnancy and lactation</u>

Bone health indices

6.49 During pregnancy and lactation, the mother provides a large amount of calcium 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).

6.50 The relationship between bone health indices and maternal serum 25(OH)D concentration during pregnancy is unclear which complicates associations between serum 25(OH)D concentration and bone health indices during this time (Brannon & Picciano, 2011).

6.51 Physiological changes that occur during pregnancy increase serum concentrations of $1,25(OH)_2D$ and DBP; serum 25(OH)D concentration, however, remains unaffected. The underlying mechanisms for these changes are not clearly understood (Brannon & Picciano, 2011).

6.52 IOM report: The IOM identified only 1 cohort study which included maternal BMD as an outcome and reported no relationship between serum 25(OH)D concentration and postpartum changes in BMD. It 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

Maternal outcomes

Intervention studies

6.53 No RCTs investigating effects of vitamin D supplementation during pregnancy/lactation on markers of maternal bone health have been published since the IOM report.

Cohort studies

6.54 A study in Turkey (Haliloglu et al., 2011) investigated the relationship between serum 25(OH)D concentration and CTX in women (n=30; mean age, 28y) receiving supplemental vitamin D_3 (10 µg/400 IU per day) during pregnancy and lactation. Mean serum 25(OH)D concentration was 19.1, 15.7, 11.1 and 7.0 nmol/L during the 1st, 2nd and 3rd trimester and postpartum 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 (Tables 8-9, Annex 2)

Intervention studies

6.55 One UK RCT (MAVIDOS[42]) has investigated effects of vitamin D supplementation during pregnancy on markers of bone health in newborn infants (Cooper et al., 2016). Pregnant women (n=1134) with a serum 25(OH)D concentration of 25-100 nmol/L at 10-17 weeks gestation were randomised to receive either 25 µg/d (1000 IU/d) of vitamin D_3 or a placebo from 14 weeks gestation[43] until delivery. Whole body BMC of neonates (n=737) was assessed within 2 weeks of birth. Mean BMC of neonates born to mothers supplemented with vitamin D_3 did not differ significantly from that of neonates born to mothers who received placebo (61.6g; 95% CI, 60.3-62.8 vs 60.5g; 95% CI, 59.3-61.7; p=0.21). Bone area or BMD also did not differ between groups. However, in a pre-specified secondary analysis, there was an interaction with season of delivery such that neonatal BMC was significantly greater for winter

[42] Maternal Vitamin D Osteoporosis Study.
[43] Or as soon as possible before 17 weeks gestation if recruited later.

births (December-February) in the vitamin D supplemented group (mean difference, 5.5g; 95% CI, 1.8-9.1; p=0.04), when maternal serum 25(OH)D concentrations in the placebo group were at their lowest. A similar winter-birth effect was observed for whole body bone area (p=0.05) and BMD (p=0.04). The authors hypothesise that vitamin D supplementation prevented the adverse effect of a decline in maternal serum 25(OH)D concentration on neonatal BMC, with the overall effect being the prevention of deficit in BMC rather than an increase. However, a significant treatment effect was not found for spring births (March-May) when maternal serum 25(OH)D concentrations were also at their lowest.

Cohort studies

6.56 Mahon et al. (2010) investigated the association between maternal serum 25(OH)D concentration and fetal femur growth in pregnant women (n=424; age, 20-34y) 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 9y of age (Javaid et al., 2006).

6.57 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 (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.

6.58 Viljakainen et al. (2010) investigated associations between serum 25(OH)D concentrations of Finnish mothers (n=125; age, 20-40y) 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.

6.59 Young et al. (2012) examined the relationship between maternal serum 25(OH)D concentration (and calcium intake) on fetal bone growth in pregnant adolescent girls (n=171; mean age, 17y) 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.

6.60 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 - Pregnancy & lactation

Bone health indices

Maternal outcomes

6.61 One small cohort study reported a negative correlation between maternal serum 25(OH)D concentration and a marker of bone resorption (CTX concentration) in the 2nd and 3rd trimesters of pregnancy and the postpartum period.

Fetal/newborn outcomes

6.62 One large RCT in the UK reported no overall effect of vitamin D supplementation during pregnancy on BMC, BMD or bone area in the newborn infant but a significantly lower BMC, BMD and bone area in infants born to non-supplemented mothers in the winter (December-February).

6.63 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

6.64 IOM report: 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., 1981; 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 (Table 10, Annex 2)

Intervention studies

6.65 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 which include use of BMD rather than BMC or BMAD in growing children and not providing data on power calculations for the sample size required to detect a bone density difference with treatment.

6.66 A study in India (Kumar et al., 2011) investigated the effect of vitamin D_3 supplementation (35 µg/1400 IU per wk for 6 months) on growth (secondary outcome[44]) in low birth weight term infants (n=2070; age, ≤ 48 hours). After 6 months, 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

[44] Primary outcomes were mortality and morbidity.

large loss to follow-up[45] and, since it was conducted in undernourished low birth weight infants, the findings may not be applicable to normal weight infants in the UK.

6.67 Abrams (2012) evaluated the effects of daily vitamin D_3 supplementation (10 µg/400 IU for 3 months) on BMC/BMD in Hispanic and non-Hispanic white infants (n=49; age, 1 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 first 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.

6.68 Holmlund-Suila et al. (2012) evaluated the effects of different daily supplemental doses of vitamin D_3 (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 - Infants (up to 12 months)

Bone health indices

6.69 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 BMC, BMD or 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 (1-3y)

Bone health indices

6.70 IOM report: The IOM did not consider this age group separately.

Evidence considered

6.71 No intervention or cohort studies examining the relationship between serum 25(OH)D concentration and BMD/BMC in this age group could be identified.

6.72 A cross-sectional analysis of children in Canada (n=488; age, 1.8-6y) reported that a plasma 25(OH)D concentration > 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 - Children (1-3y)

Bone health indices

6.73 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.

[45] Anthropometric data were available for only 62% of original sample.

Children (4-8y) and adolescents (4-17y)

Bone health indices

6.74 IOM report: In the IOM report early childhood was defined as 4-8y and puberty/adolescence was defined as 9-13y and 14-18y. 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 (Tables 11-13, Annex 2)

Systematic review and meta-analysis

6.75 A systematic review and meta-analysis of 6 intervention studies (Winzenberg et al., 2011) examined the effect of vitamin D_3 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, or lumbar spine. 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.

6.76 There are a number of limitations in this meta-analysis which limit interpretation of the results: the studies were heterogeneous in terms of sample size and ethnicity; one study administered vitamin D fortified milk rather than vitamin D supplements (Du et al., 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

6.77 Park et al. (2010) reported no effect of daily vitamin D_3 supplementation (25 µg/1000 IU) on calcium absorption or skeletal retention in girls (n=11; age 12-14y). 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.

6.78 Molgaard et al. (2010) reported that daily vitamin D_3 supplementation (5 or 10 µg/200 or 400 IU) for 12 months had no effect on indices of bone health in girls (n=221; age, 11-12y). However, following stratification by the FokI VDR gene polymorphism, whole body BMD (p=0.007) and BMC (p=0.048) increased in a subgroup with the FF VDR (but not the Ff or ff VDR) genotype, indicating an influence of VDR genotype.

6.79 Ghazi et al. (2010) found no effect of vitamin D_3 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, 14-20y).

6.80 Ward et al. (2010) reported no effect of vitamin D_2 supplementation (4 doses of 3750 µg/150,000 IU over 1 year) on BMD and BMC in adolescent girls (n=69; age. 12-14y; 88% of South Asian origin).

6.81 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, 14-15y) randomised to receive either vitamin D_2 (7500 µg/300,000 IU) or placebo 4 times/year for 1 year; all participants also received calcium (250 mg/d). Median (IQR) 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. There was no significant difference between the two groups in bone outcome measures.

Muscle strength and function

6.82 IOM Report: Muscle strength and function in children and adolescents was not considered.

Evidence considered since IOM

Intervention studies

6.83 Ward et al. (2010) examined the effect of vitamin D_2 supplementation (4 doses of 3750 µg/150,000 IU over 1 year) on muscle function in adolescent girls (n=69; age, 12-14y; 88% of South Asian origin). Mean baseline serum 25(OH)D concentration increased significantly in the intervention group (18.1 to 56 nmol/L) but not in the control group (17.9 to 15.7 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 baseline concentrations. There were no improvements in muscle force or power.

Summary - Children(4-8y) and adolescents (4-17y)

Bone health indices

6.84 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.

6.85 The majority of subsequent intervention studies did not find a beneficial effect of vitamin D supplementation on bone health indices in children and adolescents. Out of 5 studies, none reported an effect of vitamin D supplementation on bone health indices.

Muscle strength and function

6.86 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 under 50y

Bone health indices

6.87 IOM Report: 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 (Table 14, Annex 2)

Intervention studies

6.88 A 1-year RCT (Islam et al., 2010) reported a beneficial effect ($p < 0.001$) of vitamin D supplementation on femur BMD and BMC in premenopausal women in Bangladesh (n=200; age, 16-36y). Participants received daily vitamin D either alone (10 µg/400 IU), with calcium (600 mg), with calcium plus a multiple micronutrient (MMN) supplement, or placebo. Mean baseline serum 25(OH)D concentration was 36 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 + calcium + MMN supplemented groups (increase of 32·2, 32·4, 28·8 nmol/L respectively) but not in the placebo group (increase of 0·6 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.

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

Muscle strength and function

6.90 IOM Report: Muscle strength and function in adults under 50y was not considered.

Evidence considered since IOM report (Table 15, Annex 2)

Systematic review and meta-analysis

6.91 Tomlinson et al. (2015) 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, 24y). 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 D_3, or vitamin D_3 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

6.92 No cohort studies could be identified.

Stress fracture prevention

6.93 Stress fractures are caused by repetitive sub-maximal loading of bone which ultimately results in a decrease in the intrinsic ability of the bone 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%.

6.94 IOM Report: 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 (Tables 16-18, Annex 2)

Intervention studies

6.95 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 inversely correlated with fracture risk; participants who exercised ≥ 3 times/week 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 (Rauh et al., 2006; Shaffer et al., 2006).

Cohort studies

6.96 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; age, 18-30y) (Dao et al., 2015). 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 in stress fracture cases than controls (-6.6 nmol/L; 95% CI, -14.5 to 1.3; $p=0.1$).

6.97 A subsequent cohort study (Davey et al., 2016) which prospectively followed Royal Marine (RM) recruits (n=1082 males; age, 16-32y) through a 32 week training programme reported that the Odds Ratio of stress fracture for recruits with baseline serum 25(OH)D concentration < 50 nmol/L compared with ≥ 50 nmol/L was 1.6 (95% CI, 1.0-2.6).

6.98 Associations between serum 25(OH)D concentration and reduced stress fracture risk in observational studies could be confounded by the association observed between exercise and reduced stress fracture risk (Lappe et al., 2008). People who regularly exercise are likely to spend more time outdoors and have higher serum 25(OH)D concentration as a consequence of greater UVB exposure.

6.99 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 - Adults < 50y

Bone health indices

6.100 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 & function

6.101 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 fractures

6.102 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.

6.103 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.

6.104 The evidence in non-military population groups is sparse and inconsistent.

Adults 50y and above

Bone health indices

6.105 IOM Report: 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 (Tables 19-21, Annex 2)

Systematic review

6.106 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, 59y[46]) in 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 suggested this effect could have been artifactual or a chance finding. Subgroup analysis showed that age, study duration or administration of calcium did not affect outcomes.

[46] Average age was < 50 y in 6 studies (n=871).

Intervention studies

6.107 A 3-year randomised population based open trial in Finland (Karkkainen et al., 2010) examined whether daily vitamin D (20 µg/800 IU) and calcium (1000 mg) supplementation could reduce bone loss in postmenopausal women (n=593; age, 65-71y). The control group received no intervention. Mean serum 25(OH)D concentration at baseline was 50.1 and 49.2 nmol/L in the intervention and control groups respectively and 74.6 nmol/L and 55.9 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 women[47] 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 lumber spine, however, was greater in the intervention group (+0.67% vs +0.76%, p=0.03).

6.108 A 1-year RCT in Scotland (Macdonald et al., 2013) compared the effect of 2 different doses of vitamin D_3 supplementation (10 µg/400 IU or 25 µg/1000 IU daily) and placebo on BMD in postmenopausal women (n=305; mean age, 64.6y). Mean BMD loss at the hip was significantly less in the group assigned to 25 µg (1000 IU) of vitamin D_3 ($p < 0.05$) compared with the groups assigned to 10 µg (400 IU) vitamin D_3 or placebo. There were no differences in markers of bone metabolism (P1NP, CTX).

Cohort studies

6.109 A prospective study in six US centres with 4 years follow-up (Ensrud et al., 2009) reported an inverse association (p trend = 0.01) between baseline serum 25(OH)D concentration and BMD loss rates (hip and trochanter) in community dwelling men (n=1279; mean age, 73y). 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 ≥ 75y compared to < 75y. No association was found between serum 25(OH)D concentration and rate of hip bone loss among men < 75y.

Fracture prevention

6.110 IOM Report: The IOM reported that associations between serum 25(OH)D concentration and risk of fractures were inconsistent in the age group 51-70y. For the age group ≥ 71y, it concluded that supplementation with vitamin D_2 or D_3 did not reduce the risk of fractures but vitamin D (mainly vitamin D_3) 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 (Tables 22-24, Annex 2)

Meta-analysis

6.111 A meta-analysis on the efficacy of oral vitamin D supplements in preventing non-vertebral and hip fractures among adults > 65y included data from 12 RCTs (n=42,279) on non-vertebral fractures and 8

[47] Those who took at least 80% of their supplementation.

RCTs (n=40,886) on hip fractures (Bischoff-Ferrari et al., 2009b). 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/d) 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%).

6.112 Bolland et al. (2014) conducted a trial sequential meta-analysis[48] 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, 53-89y), 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).

6.113 A Cochrane review 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 (Avenell et al., 2014). Vitamin D alone (in the forms and doses tested) vs placebo or no treatment had no effect on: hip fracture (RR=1.12; 95% CI, 0.98-1.29; 11 trials; n=27,693); non-vertebral fractures (RR, 1.05; 95% CI, 0.96-1.14; 12 trials; n=22,930;); vertebral fractures (RR=1.03; 95% CI, 0.76-1.39; 6 trials, n=11,396); or any new fracture (RR=1.03; 95% CI, 0.96-1.11; 15 trials; n=28,271;). Vitamin D plus calcium was no more effective than calcium alone for: hip fracture (RR=0.84; 95% CI, 0.63-1.13; 7 trials, n=7411); any non-vertebral fracture (RR=0.96; 95% CI, 0.76-1.16; 6 trials, n=3336); and vertebral fracture (RR=0.14; 95% CI, 0.01-2.77; 2 trials, n=2681). Vitamin D plus calcium vs placebo or no treatment resulted in a statistically significant reduction in: risk of hip fracture (RR=0.84; 95% CI, 0.74-0.96; 9 trials; n=49,853); incidence of new non-vertebral fractures (RR=0.86; 95% CI, 0.78-0.96; 8 trials; n=10,380); incidence of any fracture (RR=0.95; 95% CI, 0.9-0.99; 10 trials, n=49,976). There was evidence of a statistically significant preventive effect of vitamin D plus calcium vs placebo or no treatment on clinical vertebral fractures (RR=0.89; 95% CI, 0.74-1.09; 4 trials, n=42,185).

Intervention studies

6.114 Sanders et al. (2010) examined the effect of a single high annual dose of vitamin D_3 (12,500 µg/500,000 IU) for 3-5 years on fracture reduction in community dwelling women in Australia (n=2256; median age, 76y). They 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 6.144).

Cohort studies

6.115 A nested case-control study within a prospective cohort study in the US (Cauley et al., 2010) examined associations between serum 25(OH)D concentration and fracture risk in men aged ≥ 65y 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=1608). 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).

[48] 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%.

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.

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

6.117 A cohort study in Japan of community dwelling women (n=773; mean age, 74.6y) followed up for 6 years (Nakamura et al., 2011), reported that the adjusted hazard ratios 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 hazard ratio 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 hazard ratio 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).

6.118 A cohort study in the USA, which followed community dwelling white and black participants (n=2614; age, > 70y) 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).

6.119 A cohort study which followed healthy postmenopausal women in Saudi Arabia (n=912; mean age, 61y) for 5 years (Rouzi et al., 2012), 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).

Muscle strength and function

6.120 IOM Report: 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. The IOM concluded that, overall, data from RCTs suggested that vitamin D doses of at least 20 μg/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 (Tables 25-27, Annnex 2)

Systematic reviews & meta-analyses

6.121 A systematic review and meta-analysis of 13 RCTs (n=2,268; mean age, 78y) assessed the efficacy of vitamin D supplementation on muscle strength, gait and balance (Muir & Montero-Odasso, 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 concentration (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).

6.122 Stockton et al. (2011) conducted a meta-analysis of 17 RCTs (n=5072). Participants were aged > 60y in most studies but 2 studies included younger adults (50-79y & 31.6 ± 4.8y). 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 2 studies in which the mean baseline serum 25(OH)D concentration was < 25 nmol/L reported a significant improvement in hip muscle strength (standardised mean difference, 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., 2005b) and the other in patients with Alzheimer's disease (Sato et al., 2005a). 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, 31.6y) 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) recruited hospitalised patients and found no significant effect of vitamin D supplements on strength score (derived from functional activities).

6.123 Another systematic review and meta-analysis (Beaudart et al., 2014) included 30 RCTs (n=5,615; mean age, 61 y). Supplementation consisted of vitamin D alone in 14 studies and combined with calcium in 16 studies; 4 studies used vitamin D analogues (alfacalcidol and $1,25(OH)_2D$). A small but statistically significant positive effect of vitamin D supplementation on global muscle strength was reported (standardised mean difference=0.17; 95% CI, 0.03-0.31; p=0.02) but there was significant heterogeneity (p< 0.001; I^2, 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 ≥ 65y (standardised mean difference, 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

6.124 Lips et al. (2010) compared the effect of a weekly vitamin D_3 supplement (210 µg/8,400 IU) or placebo for 16 weeks on postural stability and muscle strength in adults ≥ 70y 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 showed a treatment difference in participants with baseline sway ≥ 0.46 cm. In this cohort (n=31), postural sway was improved in the vitamin D supplemented group compared with the placebo group (p=0.047). In participants with baseline sway < 0.46 cm (n=179), there was no difference between treatment groups.

6.125 Knutsen et al. (2014) compared the effect of daily vitamin D_3 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; age, 18-50y) 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/d) vitamin D and to 52 nmol/L in the group supplemented with 25 µg/d (1000 IU/d). Vitamin D supplementation had no significant effect on jump height, handgrip strength or chair-rising test in any group.

6.126 A small RCT in Australia (n=26; mean age, 69y) with the primary aim of assessing the effect of 50 µg/d (2000 IU/d) of vitamin D_3 for 10 weeks on neuroplasticity, also measured muscle strength and function (Pirotta et al., 2015). 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 (49 nmol/L at baseline). Compared to baseline, there was a significant 8-11 % increase in muscle strength in the vitamin D supplemented group ($p < 0.05$) 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

6.127 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, 62y; 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 ($p=0.027$).

6.128 Another prospective study (Menant et al., 2012) of community dwelling adults in Australia (n=463; age, 70-90y) 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[49]=1.94; 95% CI, 1.19–3.15; $p=0.008$).

6.129 A longitudinal analysis in the US (North Carolina) of community dwelling people (n=988; age, 77-100y) 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 (Houston et al., 2011). 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).

6.130 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=1471; 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.

6.131 Another prospective study in Hong Kong followed community dwelling men (n=714; mean age, 73y) 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.

[49] Incident rate ratio.

6.132 Michael et al. (2011) assessed measures of muscle strength in postmenopausal women (n=532) 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 baseline serum 25(OH)D concentration was 48.2 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.

6.133 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 USA (n=2641; age, 71–80y). 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 ages 2 and 4y ($p < 0.01$). 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.

Falls

6.134 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.

6.135 IOM Report: The IOM report considered *'falls'* and *'physical performance'* together. 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.

6.136 The IOM considered 2 meta-analyses by Bischoff-Ferrari (2004, 2009a). 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 1α-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 40.5-65[50] nmol/L after supplementation. A secondary analysis which included 5 additional studies (n=1001) in a sensitivity analysis reported a smaller but still significant effect size (corrected RR=0.87, 95% CI, 0.80-0.96).

6.137 The 2009 meta-analysis included 8 RCTs (n=2426) in the primary analysis but analysed separately the 2 trials which used 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

[50] Concentrations declined because intervention was active D.

heterogeneity among trials for dose of vitamin D and achieved serum 25(OH)D concentration. Vitamin D doses of 17.5-25 µg/d (700-1000 IU/d) reduced fall risk (pooled RR=0.81, 95% CI, 0.71-0.92) but doses of 5-10 µg/d (200-400 IU/d) 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).

6.138 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 criteria and inclusion of one study that did not 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 by the IOM reported a null effect of vitamin D supplementation on falls.

Evidence considered since IOM report (Tables 28-30, Annex 2)

Systematic reviews and meta-analyses

6.139 A Cochrane review (Cameron et al., 2012) which assessed the effect of vitamin D supplementation on fall prevention in adults aged > 65y[51] in nursing care facilities and hospitals 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 trials[52]; n=5186). Mean baseline serum 25(OH)D concentration in the included studies ranged from 23 to 59 nmol/L.

6.140 A second Cochrane review investigated the effect of vitamin D supplementation on fall prevention in community dwelling adults aged ≥ 60y[53] (Gillespie et al., 2012). Overall, vitamin D did not reduce rate of falls (RaR[54]=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 concentration[55] reported a greater reduction in rate of falls (RaR=0.57; 95% CI, 0.37-0.89; 2 trials[56]; 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 trials[57]; 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).

6.141 A meta-analysis (Kalyani et al., 2010) of 10 RCTs in community dwelling and institutionalised adults ≥ 60y (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 < 80y; when calcium was co-administered, when vitamin D_3 was used at doses > 20 µg/d (800 IU/d), 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.

[51] Trials were also included if the mean age was > 65y.
[52] 1 trial tested a vitamin D supplement that included vitamin D plus calcium (Grieger, 2009).
[53] Trials also included if mean age minus 1 standard deviation was more than 60 years.
[54] Rate ratio.
[55] 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).
[56] Dhesi *et al* (2004); Pfeifer *et al* (2000).
[57] Dhesi *et al* (2004); Pfeifer *et al* (2000); Pfeifer *et al* (2009); Prince *et al* (2008).

6.142 Another meta-analysis (Murad et al., 2011) of 26 trials (n=45,782; mean age, 76y) 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%). Subgroup analysis showed risk reduction was greater in participants who were considered vitamin D deficient[58] 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 exaggerated the estimates of risk reduction.

6.143 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, 22-75 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 analysis[59] with a risk reduction threshold of 15%. In the 20 RCTs included in the analysis, the effect estimate for vitamin D supplementation (+/- calcium) on falls lay within the futility boundary, indicating that it does not alter the relative risk of falls by 15% or more.

Intervention studies

6.144 An RCT of community-dwelling women (n=2256; median age 76y) 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 explain why effects differed from those observed with daily supplementation.

6.145 In another RCT (Bischoff-Ferrari et al., 2016), community-dwelling men and women (n=200; mean age, 78y with a prior fall) were randomised to receive a monthly dose of either 600 µg (24,000 IU) vitamin D_3, 1500 µg (60,000 IU) vitamin D_3, or 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3 for 12 months. The primary outcome was improvement in lower extremity function and achieving serum 25(OH)D concentrations ≥ 75 nmol/L; a secondary outcome was monthly reported falls. The groups receiving 1500 µg (60,000 IU) vitamin D_3 and 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3 were more likely to achieve serum 25(OH)D concentrations ≥ 75 nmol/L than the 600 µg (24,000 IU) vitamin D_3 alone group. There was no difference between groups in lower extremity function but the incidence of falls was significantly higher in the 1500 µg (60,000 IU) vitamin D_3 group (66.9%; 95% CI, 54.4-77.5%) and

[58] 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.
[59] 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).

the 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3 group (66.1%; 95% CI, 53.5-76.8%) compared with the 600 µg (24,000 IU) vitamin D_3 group (47.9%; 95% CI, 35.8-60.3%) (p=0.048).

Cohort studies

6.146 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, 70-90y) 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

6.147 A study in Italian adults (n=259; mean age, 85y; 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; mean age, 54.3y) and the Osteoporosis and Ultrasound Study (OPUS; n=1970; mean age, 66.9y). An increase in falls risk with the BB *Bsm1* genotype was found 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 - Adults ≥ 50y

Bone health indices

6.148 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.

6.149 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.

6.150 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

6.151 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 reported a beneficial effect of vitamin D plus calcium on fracture prevention.

6.152 One RCT reported an increased risk of fracture with a single high annual dose of vitamin D (12,500 µg/500,000 IU).

6.153 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

6.154 Three meta-analyses of RCTs reported a beneficial effect of vitamin D supplementation on muscle strength and function in adults ≥ 50y 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.

6.155 Three subsequent RCTs were largely unsupportive of an effect of vitamin D supplementation on muscle strength.

6.156 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

6.157 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 ≥ 50y 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; I meta-analysis found no beneficial effect of vitamin D supplementation with or without calcium on risk of falls.

6.158 One RCT reported that a single high annual dose of vitamin D (12,500 µg/500,000 IU) increases the risk of falls. Another reported an increased risk of falls with a monthly vitamin D dose of 1500 µg (60,000 IU) or 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3 compared with 600 µg (24,000 IU) vitamin D_3 alone.

6.159 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.

6.160 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 in 2 cohorts.

Conclusions - vitamin D and musculoskeletal outcomes

Rickets

6.161 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, in the majority of studies, individual or mean serum 25(OH)D concentration was < 25 nmol/L (the current threshold associated with increased risk of vitamin D deficiency) in children with rickets.

6.162 It is not known whether the sole cause of rickets was 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 at or above 25 nmol/L might be explained by calcium deficiency.

6.163 Although the risk of rickets increases at serum 25(OH)D concentrations < 25 nmol/L, this concentration is not a clinical threshold diagnostic of the disease and most children in the general population who have a serum 25(OH)D concentration < 25 nmol/L will not develop rickets.

Osteomalacia

6.164 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; and 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

6.165 Bone health indices (BMC/BMD/biochemical markers of bone formation and resorption) were considered in 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 ≥ 50y. 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 1-3y and adults aged < 50y was too small to draw any conclusions.

Fracture prevention

6.166 Data on vitamin D supplementation and fracture prevention in adults aged ≥ 50y are mixed but suggest that vitamin D plus calcium is more effective in reducing fracture risk than vitamin D alone. On balance, vitamin D supplements had no beneficial effect on fracture risk in adults aged ≥ 50y.

6.167 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

6.168 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 aged < 50y with a mean serum 25(OH)D concentration < 30 nmol/L.

6.169 In adults aged ≥ 50y, evidence is mixed but, overall, was 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 was 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

6.170 Evidence on vitamin D and falls is mixed but, overall, was suggestive of a beneficial effect of vitamin D supplementation in reducing fall risk in adults ≥ 50y with mean baseline serum 25(OH)D concentrations ranging between < 25 and around 80 nmol/L.

6.171 Two RCTs reported that high-dose vitamin D supplementation increased fall risk. The supplementation dose was administered annually in 1 RCT (12,500 µg/500,000 IU) and monthly in the other (1500 µg/60,000 IU or 600 µg/24,000 IU vitamin D_3 + 300 µg 25(OH)D_3). Serum 25(OH)D concentrations achieved in these studies ranged from 75 to 90 nmol/L. High doses of vitamin D, administered annually or monthly, may have different effects from daily supplementation at lower doses.

Non-musculoskeletal health outcomes (Tables 31-54, Annex 2)

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

6.172 IOM Report: In relation to maternal non-skeletal outcomes, the IOM report considered pre-eclampsia/pregnancy induced hypertension[60] (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, the IOM considered birth weight but noted conflicting evidence from RCTs and observational studies.

Evidence considered since IOM report (Tables 31-36, Annex 2)

Maternal non-skeletal reproductive outcomes

6.173 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, associated with a rise in DBP (Kovacs, 2008).

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

Systematic reviews

6.175 A Cochrane systematic review (De-Regil et al., 2016) examined vitamin D supplementation alone (9 trials; n=1251) or combined with calcium (6 trials) on maternal outcomes. Vitamin D alone (2 trials; n=219) had no effect on pre-eclampsia risk (RR=0.52; 95% CI, 0.25-1.05) but vitamin D plus calcium (3 trials; n=1114) significantly lowered risk of pre-eclampsia (RR=0.51; 95% CI, 0.32-0.80). Vitamin D (alone or with calcium) had no effect on risk of gestational diabetes. However most trials were of low methodological quality, sample sizes were small (40-400) and there was considerable variation in vitamin D doses and regimens (5-50 µg/200-2000 IU daily; 875 µg/35,000 IU weekly; or 5000 µg/200,000 IU to 15,000 µg/600,000 IU single doses). The review did not include any information or consideration about mean baseline 25(OH)D concentrations in the included studies.

6.176 A systematic review of observational studies (Harvey et al., 2014) identified 11 studies (6 case-control, 4 cohort, 1 cross-sectional) that had examined the effect of maternal serum 25(OH)D concentration during pregnancy on PIH. Five 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. Eight studies (4 case-control, 1 cross-sectional, 3 prospective) examined associations between maternal serum 25(OH)D concentration and risk of gestational diabetes. The findings were inconsistent but the majority of studies found no association.

[60] Pre-eclampsia, a serious complication that occurs during pregnancy, is characterised by high blood pressure and protein in the urine; PIH is characterised by high blood pressure without the presence of protein in the urine.

Intervention studies

6.177 An RCT in the US (Hollis et al., 2011) not included in the above review (De-Regil et al., 2016) reported no effect of daily vitamin D_3 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. (2013a) also found no difference in comorbidities of pregnancy by treatment group (Wagner et al., 2013b) but reported 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.

Non-skeletal outcomes in the newborn

Effect of maternal serum 25(OH)D concentrations in pregnancy on neonate/infant "stores"

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

6.179 The correlation between maternal and fetal serum 25(OH)D concentrations 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 serum 25(OH)D concentration in pregnancy was sufficient, but can only be considered an approximation.

Neonatal hypocalcaemia

Intervention studies

6.180 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.

6.181 The study by Cockburn et al. (1980) was not randomised (allocation by hospital ward) but study groups were treated concurrently. Participants (n=1139) received a vitamin D_2 supplement (10 µg/400 IU) or placebo daily from the 12th week of pregnancy until delivery. Mean maternal plasma 25(OH)D concentration at delivery was 43 nmol/L in supplemented women and 33 nmol/L in controls. Corresponding 25(OH)D concentrations were 28 and 20 nmol/L in umbilical venous blood and 35 and 20 nmol/L in 6th day capillary samples. Neonatal hypocalcaemia[61] occurred in 6% of the intervention group infants and 13% of controls ($p < 0.005$). Out of 61 infants who had their teeth examined in their 3rd year, a significantly higher incidence of dental enamel hypoplasia was observed

[61] Defined as plasma calcium < 1.85 mmol/L.

in those born to control mothers and who had been hypocalcaemic compared to those born to supplemented mothers.

6.182 Brooke et al. (1981) randomly allocated women of South Asian ethnic origin (n=126) to receive vitamin D_2 (25 µg/1000 IU daily) or placebo in a double-blind design. Five control infants but no treatment group infants developed symptomatic hypocalcaemia[62]. At delivery, between group differences in mean maternal plasma 25(OH)D concentration (168 nmol/L, intervention group; 16 nmol/L, control group) and mean umbilical venous plasma 25(OH)D concentration (138 nmol/L, intervention group; 10 nmol/L, control group) were very striking and have not been replicated in any subsequent studies.

6.183 Delvin et al. (1986) randomly assigned pregnant women (n=40) in the 6th month of pregnancy to receive either vitamin D_3 (25 µg/1000 IU daily) 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 ($p < 0.05$).

Birth weight and length, small for gestational age

Systematic reviews

6.184 A Cochrane systematic review of randomised trials (De-Regil et al., 2016) reported no difference in birth weight of infants born to vitamin D supplemented women compared to those of mothers who were not supplemented (5 trials; n=715). Four trials (n=638) suggested a trend for higher birth length (p=0.06) among infants whose mothers had taken vitamin D supplements during pregnancy compared to no treatment/placebo but there was considerable heterogeneity (I^2 = 77%) and a significantly higher mean head circumference at birth (mean difference=0.43; 95% CI, 0.03-0.83). Three trials (n=477) suggested that vitamin D supplemented women had a lower risk of a preterm birth (RR=0.36; 95% CI, 0.14-0.93); however vitamin D plus calcium supplements (3 trials; n=798) during pregnancy appeared to increase risk of preterm birth (RR=1.57; 95% CI, 1.02-2.43). The authors noted that most of the trials were of low methodological quality with many studies at high risk of bias for blinding and attrition rates and advised caution in the interpretation of the findings.

6.185 Another systematic review (Harvey et al., 2014) identified 9 intervention studies (n=40-350); only 1 of these was double-blinded and placebo controlled (Brooke et al., 1980). Three studies (all from India) reported birth weight was significantly greater in infants of vitamin D supplemented mothers but the 3 studies from the UK (Brooke et al., 1980; Congdon et al., 1983; Yu et al., 2009) did not. 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 D_2 supplementation (25 µg/1000 IU) during the last trimester while the other (Marya et al., 1988) reported significantly higher birth length ($p < 0.001$) in infants of women supplemented with vitamin D_3 (15,000 µg/600,000 IU in 7th and 8th month of gestation). Two trials (Brooke et al., 1980; Yu et al., 2009) found no effect of vitamin D supplementation on risk of offspring being born small for gestational age[63] (SGA).

6.186 The Harvey et al. (2014) review also included 19 observational studies on the association between maternal serum 25(OH)D concentration and 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. Out of 8 cohort studies, none found an association between

[62] Defined as plasma calcium < 1.8 mmol/L)
[63] Both trials defined SGA as infants born below the 10th percentile for birth weight.

maternal serum 25(OH)D concentration and offspring birth length. However one Dutch prospective cohort study (n=3730), 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 (Leffelaar et al., 2010). Three out of 7 studies (4 cohort, 2 case-control & 1 cross-sectional study) that assessed the relationship between SGA and maternal serum 25(OH)D concentration reported a significant association: 2 reported an inverse association while 1 (Bodnar et al., 2010), a nested case control study in the USA of black and white women (from < 16 wks gestation), observed a U-shaped association among white mothers with a significantly increased risk at 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., 2006; Morley et al., 2009) in an Australian cohort, might explain differences between populations.

Intervention studies

6.187 An RCT in the USA, of pregnant women (n=257; 12-16 weeks gestation) supplemented daily with vitamin D_3 (50 µg/2000 IU or 100 µg/4000 IU), reported no differences in neonatal birth weight, gestation or health (Wagner et al., 2013a). 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.

6.188 Another very large RCT (n=1134) in the UK (the MAVIDOS study), that examined the effect of daily vitamin D supplementation (25 µg/1000 IU) during pregnancy (baseline serum concentration between 25-100 nmol/L) on neonate bone health (see paragraph 6.55), reported no difference between the intervention and placebo groups in neonate birth weight, birth length and head circumference (Cooper et al., 2016).

Observational studies

6.189 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 second trimester plasma 25(OH)D concentration was lower in black (46 nmol/L) compared to white (62 nmol/L) mothers. Mean cord plasma 25(OH)D concentration was also lower in black (31 nmol/L) compared to white (51 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).

Later growth and development of the offspring

Cognitive and psychological development

6.190 A prospective cohort study in Southampton, did not find an association between maternal serum 25(OH)D concentration in pregnancy and any test of cognitive performance or psychological health to the age of 9y (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 17y although there was a significant association between low serum 25(OH)D concentration in pregnancy and offspring language delay at ages 5 and 10y (Whitehouse et al., 2012). Risk was approximately doubled in mothers with serum 25(OH)D concentration < 46 nmol/L compared to > 70 nmol/L in pregnancy.

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

Later growth

6.192 A single RCT (Brooke et al., 1981) observed associations between ponderal and linear growth after birth (for 1 year) with maternal serum 25(OH)D concentration in pregnancy. Infants of mothers who received daily vitamin D_2 supplementation (25 µg/1000 IU during last trimester) compared to those of mothers who received placebo, were significantly heavier throughout the first year of life and longer at 9 and 12 months of age but there was no effect on head circumference at any stage of infancy.

6.193 In contrast, a prospective cohort study in Southampton (see paragraph 6.190) observed a significantly greater head circumference at age 9y 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

6.194 Data from the prospective study by Gale et al. (2008) found no association between maternal serum 25(OH)D concentration in pregnancy and increased cardio-respiratory risk at 9y of age. However, the offspring of mothers whose serum 25(OH)D concentration in pregnancy exceeded 75 nmol/L were at greater risk of eczema at age 9m and asthma at age 9y.

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

Non-skeletal health outcomes in the mother

6.195 A systematic review suggests that women who receive vitamin D supplements combined with calcium (3 trials), but not vitamin D alone (2 trials), may have a lower risk of pre-eclampsia compared to those receiving no treatment. Data from 2 trials indicate no effect of vitamin D supplementation on gestational diabetes.

6.196 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 baby

6.197 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.

6.198 Evidence from 3 intervention trials indicates a reduction in neonatal hypocalcaemia incidence with maternal vitamin D supplementation in pregnancy.

6.199 Evidence from systematic reviews of intervention trials on effects of vitamin D supplementation during pregnancy on neonate birth weight and length is inconsistent and most of the included trials had a small sample size and methodological limitations. One systematic review indicates that vitamin D supplementation does not have beneficial effects on infant birth weight, birth length, head circumference or risk of offspring being SGA. Another suggests that vitamin D supplementation during pregnancy may reduce the risk of preterm birth and increase head circumference. A subsequent large RCT in the UK reported that vitamin D supplementation had no effect on birth weight, birth length or head circumference. Evidence from observational studies is inconsistent**.**

6.200 Findings from observational studies on associations between maternal serum 25(OH)D concentration and cognitive and psychological development of the offspring are inconsistent.

Cancers

6.201 Ecological studies have reported an increase in risk of several cancers with increasing latitude (WHO/IARC, 2008)[64]. 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 is supported by some cell culture studies (Cross et al., 1991; Halline et al., 1994) which found that $1,25(OH)_2D$ inhibited growth of malignant cell culture lines and reduced tumour development and growth in animal studies (Mokady et al., 2000; Tangpricha et al., 2005).

6.202 IOM Report: The IOM reviewed the evidence on vitamin D and risk of all cancers, breast cancer, colorectal cancer and prostate cancer.

6.203 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, reported 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 (Tables 37-39, Annex 2)

RCTs

6.204 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=45,151; 4333 cases) reported that vitamin D supplementation (10-27.5 µg/400-1100 IU per day) over 2-7 years had no effect on cancer risk (RR=1.00; 95% CI, 0.94-1.06; p=0.998) with no evidence of heterogeneity (I^2=0%).

6.205 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/800-1100 IU daily) on risk of breast cancer (n=5372; RR=1.11; 95% CI, 0.74-1.68).

6.206 The AHRQ update (Newberry et al., 2014) did not identify any new RCTs on the effect of vitamin D supplementation on risk for total cancer.

Cohort studies

6.207 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.

6.208 The AHRQ update (Newberry et al., 2014) identified 2 cohort studies of the association between serum 25(OH)D concentration and all-cause cancer. One of these (de Boer et al., 2012) followed white

[64] The International Agency for Research on Cancer is the specialised cancer agency of the World Health Organization.

adults (n=1621; mean age, 74y) in the US for a median of 11 years and reported no association between seasonally adjusted serum 25(OH)D concentration and cancer. The other study (Ordonez-Mena et al., 2013) followed adults in Germany (n=9580; age, 50-74y) for more than 8 years and reported an association between low serum 25(OH)D concentration (season specific ranges, 30-36 nmol/L) and increased risk for any cancer in men but not in women.

6.209 *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). Another meta-analysis of 9 studies (Chung et al., 2011) reported that each 10 nmol/L increase in pre-diagnosis 25(OH)D concentration was associated with a 6% reduction in risk of colorectal cancer (OR=0.94; 95% CI, 0.91-0.97; p<0.05). Out of 3 subsequent studies, 1 (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., 2015) reported a significantly lower risk for participants in the highest category of serum 25(OH)D concentration (see Table 39 in Annex 2 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).

6.210 *Breast cancer*: A meta-analysis of 14 studies (Kim & Je, 2014) reported that the risk of breast cancer incidence was non-significantly lower for participants in the highest (> 77 nmol/L) vs lowest quantile (< 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.

6.211 *Prostate cancer*: A meta-analysis of 14 studies (Gilbert et al., 2011) found a non-significant increase in prostate cancer risk 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 (Albanes et al., 2011; Meyer et al., 2013) reported that higher serum 25(OH)D concentration was associated with significantly increased risk of prostate cancer. Meyer et al. (2013) 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. (2011) 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 (≤ 16.3 nmol/L in winter; ≤ 25.9 nmol/L in summer). The other 3 studies (Brandstedt et al., 2012; Kristal et al., 2014; Schenk 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 concentration[65] (OR=0.43; 95% CI, 0.24-0.76), however there was no association with overall prostate cancer.

6.212 *Other cancers*: Less evidence is available for other cancers. No significant association with serum 25(OH)D concentration was reported for cancers of the oesophagus and stomach combined (Abnet et al., 2010), larynx and oropharynx combined (Arem et al., 2011), lung (Kilkkinen et al., 2008; Weinstein et al., 2011), endometrium (Zeleniuch-Jacquotte et al., 2010), ovary (Yin et al., 2011), kidney

[65] Plasma 25(OH)D concentrations not specified.

(Gallicchio et al., 2010; Mondul et al., 2014) or non-Hodgkin lymphoma (Purdue et al., 2010). Out of 2 studies on liver cancer (Wang et al., 2013a; Fedirko et al., 2014), 1 (Fedirko et al., 2014) reported that serum 25(OH)D concentration < 50 nmol/L vs ≥ 75 nmol/L was associated with a 49% reduced risk of liver cancer (IRR=0.51; 95% CI, 0.26-0.99). Out of 2 studies on bladder cancer (Mondul et al., 2010; Mondul et al., 2012), 1 (Mondul et al., 2010) reported that serum 25(OH)D concentration < 25 nmol/L vs ≥ 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 ≥ 50 nmol/L compared to < 25 nmol/L (Major et al., 2012; Afzal et al., 2013) and ≥ 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 studies[66]; RR=1.82; 95% CI, 1.38-2.40) and non-melanoma skin cancer (2 studies[67]; 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 (≥ 100 nmol/L) compared to lower (< 25 nmol/L) serum 25(OH)D concentration.

Summary - Cancers

6.213 The small number of RCTs have not shown an effect of vitamin D supplements on overall cancer risk.

6.214 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.

6.215 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

6.216 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 and age[68].

6.217 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 advance 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.

6.218 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 (Toda et al., 1983; Toda et al., 1985). It has been proposed (R Fraser, *personal communication,* 2011)

[66] Serum 25(OH)D concentrations (nmol/L) in top vs bottom quantile in the 4 studies were: >85 vs < 51; >78 vs <49; >37.4 vs <37; >75 vs <75.
[67] Serum 25(OH)D concentrations (nmol/L) in top vs bottom quantile in the 2 studies were: >50 vs <25; >75 vs <40.
[68] British Heart Foundation (https://www.bhf.org.uk/heart-health/conditions/cardiovascular-disease).

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.

6.219 Ecological studies have reported increased CVD mortality and hypertension at more Northern latitudes and during the winter (Grimes et al., 1996; Rostand, 1997; Zittermann 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 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.

6.220 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.

6.221 IOM Report: 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 concluded that it could not draw an inference about the efficacy of this indicator to support DRI development.

Evidence considered since the IOM report (Tables 40-41, Annex 2)

CVD

Systematic reviews and meta-analyses

6.222 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.

6.223 A systematic review of 8 randomised trials with CVD as a secondary outcome (Wang et al., 2010a) 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.

6.224 In contrast, a meta-analysis of 3 randomised placebo controlled trials (Bolland et al., 2011) reported that calcium and vitamin D increased the risk of myocardial infarction (MI) (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 MI or stroke (RR=1.16; 95% CI, 1.02-1.32; p=0.02).

6.225 Another systematic review (Ford et al., 2014) of 21 RCTs (n=13,033; mean/median age ≥ 60y; ≥ 1 year follow-up) reported that estimated HRs (95% CIs) for vitamin D compared with placebo or control for cardiac failure, MI 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.

6.226 A systematic review and meta-analysis of 9 prospective studies (4 on non-fatal CVD events and 5 on CVD mortality) reported that in 2 out of the 4 studies on CVD events, risk was significantly increased in participants with serum 25(OH)D concentration < 37 nmol/L (Grandi et al., 2010). 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 CVD 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 (HR=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).

6.227 (Pilz et al., 2011) summarised population-based prospective cohort studies examining the association between serum 25(OH)D concentration and CVD events and mortality. The studies were 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 of serum 25(OH)D concentration with increased CVD risk.

6.228 Another meta-analysis of 19 prospective cohort studies (Wang et al., 2012) reported an inverse association between baseline serum 25(OH)D concentration and CVD risk, 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).

Cohort studies

6.229 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 reported an association (Karakas et al., 2013; Kuhn et al., 2013; Perna et al., 2013; Robinson-Cohen et al., 2013). Karakas et al. (2013) found 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 (Tables 42-43, Annex 2)

Systematic reviews and meta-analyses

6.230 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.

6.231 Another meta-analysis (Wu et al., 2010) of 4 double blind RCTs in normotensive and hypertensive individuals (n=429) reported that vitamin D supplementation significantly reduced systolic blood pressure by 2.44 mm Hg (95% CI, -4.86 to -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.

6.232 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 to 1.10 mmHg) and diastolic (-0.52; 95% CI, -1.18 to 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 to -0.34 mm Hg; p=0.01) in participants with pre-existing cardiometabolic disease.

6.233 A much larger systematic review and meta-analysis of placebo-controlled RCTs with a minimum duration of 4 weeks (Beveridge et al., 2015) included 46 trials (n=4541) and obtained individual patient data for 27 trials (n=3092). Vitamin D supplementation had no effect on systolic or diastolic blood pressure in the trial level meta-analysis or in the individual patient data analysis. Sub-group analysis found no evidence of blood pressure reduction in individuals with elevated blood pressure.

6.234 The lack of consistent findings in these meta-analyses, indicate the relative weakness of the data.

6.235 Observational studies have shown an inverse association between serum 25(OH)D concentration and hypertension. A meta-analysis (Burgaz et al., 2011) of 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 vs lowest category of serum 25(OH)D concentration. In a dose-response meta-analysis, the odds ratio for a 40 nmol/L (approximately 2 SDs) increment in serum 25(OH)D concentration was 0.84 (95% CI, 0.78-0.90).

Cohort studies

6.236 A subsequent prospective cohort study of men in the US (n=1211) followed up for 15 years (Wang et al., 2013b), 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 and hypertension

6.237 Intervention studies have generally considered CVD risk as a secondary outcome; these studies, therefore, need to be interpreted with caution.

6.238 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.

6.239 Prospective cohort studies, overall, report inverse associations 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.

6.240 Meta-analyses of intervention studies on vitamin D supplementation and hypertension are inconsistent. A large systematic review and meta-analysis (46 trials) of placebo-controlled RCTs reported no effect of vitamin D supplementation on systolic or diastolic blood pressure.

6.241 Observational studies (cohort and cross-sectional) report an inverse association between serum 25(OH)D concentration and hypertension.

All-cause mortality

6.242 An effect of vitamin D on mortality risk has been observed in numerous observational studies that have reported associations between low serum 25(OH)D concentration and increased risk of several chronic diseases (including CVD and cancer).

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

Evidence since IOM report (Tables 44-45, Annex 2)

Systematic reviews and meta-analyses

6.244 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 years); 34 trials used vitamin D in combination with calcium in the intervention group. Participants in most of the trials were women aged over 70y (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 D_3 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 D_2 administered alone (8 trials; n=17,079) also had no statistically significant effect on mortality (RR=1.03; 95% CI, 0.96-1.12).

6.245 A meta-analysis of observational prospective studies (Schottker et al., 2014) which combined individual participant data from 8 cohorts in the USA and Europe (n=26,018; age, 50-79y) 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.

6.246 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).

Cohort studies

6.247 A subsequent prospective cohort study of community dwelling adults in Spain (n=328; age, 85y) 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 70-88y) 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[69] III (1988-1994) data (n=15,099; 3,784 deaths; age, ≥ 20y) with 15 years follow-up (Sempos et al., 2013), serum 25(OH)D concentration < 20 nmol/L was associated with significantly increased mortality risk (RR=1.5; 95% CI, 1.2-1.8) compared to the reference category of 75-99 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 model[70] (RR=1.5; 95% CI, 1.02-2.3) but the association was attenuated after further adjustment[71] (RR=1.4; 95% CI, 0.96-2.2).

Summary - All-cause mortality

6.248 Evidence from a systematic review of 56 randomised trials shows that vitamin D supplementation in combination with calcium reduces mortality risk but vitamin D supplementation alone does not affect total mortality.

6.249 Evidence from 2 meta-analyses of observational studies indicates 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.

Immune modulation

6.250 The human immune system involves a complex network of cells, tissues and organs that protect the body against pathogens by recognising and destroying substances that contain antigens[72]. It comprises the innate (non-specific) immune system and the adaptive (or acquired) immune system. The innate immune system represents the first line of host defence, responding to foreign antigens in a non-specific and generic way without conferring long-lasting immunity. Components of the innate immune response include physical barriers (e.g., skin and mucus), cytokines (proteins important in cell signalling) and white blood cells. The adaptive immune system is triggered against pathogens that are able to overcome innate immune defences. The adaptive immune system (includes lymphocytes and dendritic cells) is activated by Toll-like receptors (TLR)[73] which recognise components derived from pathogens. There are two types of adaptive immune responses: humoral immunity, mediated by antibodies produced by B lymphocytes, and cell-mediated immunity, mediated by T lymphocytes. The adaptive immune system is slower to respond but confers long-lasting immunity to specific antigens.

[69] The National Health and Nutrition Examination Survey (NHANES) is a survey programme designed to assess the health and nutritional status of adults and children in the United States.
[70] Age, sex, race-ethnicity, season.
[71] 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.
[72] Any foreign substance that triggers an immune response. Antigens carry marker molecules (usually proteins) that identify them as foreign.
[73] 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.

6.251 VDRs are expressed in cells of the immune system (e.g., lymphocytes, macrophages, natural killer cells) suggesting that vitamin D may have immunomodulatory effects. Low serum 25(OH)D concentration has been associated with allergic conditions (e.g., asthma and atopic conditions) and autoimmune diseases. It has been proposed that vitamin D may play a role in the development of allergic and autoimmune disease based on ecological data reporting greater prevalence of these diseases in Northern latitudes where sun exposure is lower (Ponsonby et al., 2002; Staples et al., 2003).

6.252 Autoimmune disease is caused by a dysfunction of the body's immune system and is mediated by T or B cell activation leading to tissue damage. The aetiology and pathogenesis of most autoimmune disorders remains unknown and several factors have been implicated in their development. A number of epidemiological studies have reported an association between low serum 25(OH)D concentration and asthma and atopic disorders as well as several autoimmune disorders including inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus (Kriegel et al., 2011; Antico et al., 2012).

6.253 IOM Report: The IOM considered evidence on asthma and autoimmune disease (type I diabetes, inflammatory bowel and Crohn's disease, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus). It concluded that, overall, the evidence was not consistently supportive of a causal role for vitamin D in reducing risk of asthma or autoimmune disease.

Evidence considered since IOM report (Tables 46-49, Annex 2)

Systematic reviews and meta-analyses

6.254 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 concluded that although genetic and epidemiological studies suggest a potential role of vitamin D in the prevention of autoimmune diseases there was little evidence of benefit from vitamin D supplementation.

Asthma and atopic disorders

Intervention studies

6.255 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 D_2 (20 µg/800 IU daily), a single dose of vitamin D_3 (5000 µg/200,000 IU), or no vitamin D. There was no significant difference between groups in risk of wheeze at 3y of age (HR=0.86; 95% CI, 0.49-1.50; p=0.69).

Cohort studies

6.256 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.

6.257 Tolppanen et al. (2013) examined prospective associations between serum 25(OH)D_2 and 25(OH)D_3 concentration measured in children aged 9.8y and incidence of wheezing (n=3323; 141 cases), asthma (n=3323, 464 cases) and flexural dermatitis (n=3748; 300 cases). Serum 25(OH)D_2 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)D_3 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).

6.258 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).

6.259 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).

6.260 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 7y 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

6.261 No intervention studies could be identified.

Cohort studies

6.262 A systematic review by Harvey et al. (2014) 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 5y 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.

6.263 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).

6.264 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, 1.04-2.45[74]). 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.

Autoimmune disease

Type I Diabetes Mellitus

Intervention studies

6.265 No intervention studies could be identified.

Cohort studies

6.266 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, 9m-10y) 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.

6.267 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., 2013). Non-Hispanic whites with serum 25(OH)D concentration ≥ 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.

6.268 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 (Sorensen et al., 2012). Mean serum 25(OH)D concentration in pregnant women (n=109) who delivered a child that subsequently developed type I diabetes before 15y of age 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 (≤ 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)

6.269 IBD is a group of chronic inflammatory conditions affecting 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 2 cross-sectional analyses that had evaluated serum 25(OH)D concentrations in patients with IBD (Jahnsen et al., 2002; Pappa et al., 2006).

[74] 4th quartile (25th-75th percentile: 80-152 nmol/L) vs 1st quartile (25th – 75th percentile: 15-36 nmol/L).

6.270 No studies on vitamin D and risk of IBD published after the IOM report could be identified. An RCT in Denmark assessed the effect of oral vitamin D_3 treatment on clinical relapse in patients (n=94) with Crohn's disease in remission (Jorgensen et al, 2010). Patients were randomised to receive either vitamin D_3 (30 µg/1200 IU per day) or placebo for 12 months. The mean serum 25(OH)D concentration of patients in the vitamin D treatment group increased from 69 to 96 nmol after 3 months ($p < 0.001$). The relapse rate was lower in the vitamin D_3 treated group compared with the placebo group (13% compared to 29%; p=0.06).

Multiple Sclerosis

6.271 IOM Report: 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

6.272 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

6.273 Huang & Xie (2012) and Tizaoui et al. (2015) conducted meta-analyses of case-control studies investigating the association between VDR polymorphisms and risk of MS. Huang & Xie (2012) reported that the ApaI, BsmI, FokI and TaqI polymorphisms were not associated with MS risk. Tizaoui et al. (2015) reported a significant association between the ApaI 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 ApaI does not.

6.274 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

6.275 IOM Report: 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

Evidence considered since IOM report

Intervention studies

6.276 Postmenopausal women (n=36,282) in the Women's Health Initiative Study of calcium and vitamin D were randomised to receive vitamin D_3 (10 µg/400 IU) plus calcium (1000 mg) or placebo daily (Racovan et al., 2012). 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 vitamin D and placebo groups (HR=1.04, 95% CI, 0.76-1.41).

Systemic lupus erythematosus (SLE)

6.277 IOM Report: The IOM did not identify any RCTs or meta-analyses for this indicator. Observational studies which suggested an association between vitamin D and SLE showed variability in the 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.

6.278 No further studies published after the IOM report, on serum 25(OH)D concentration and risk of SLE, could be identified.

Summary - Immune modulation

6.279 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.

6.280 Findings from cohort studies of atopic disorders are inconsistent.

6.281 A large systematic review (219 intervention, prospective and cross-sectional studies) reported that vitamin D supplementation has little effect on risk of developing autoimmune disease.

6.282 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.

6.283 Evidence to link vitamin D and MS is largely observational and inconsistent. Genetic studies suggest associations between the ApaI and FokI VDR polymorphisms and MS risk but the role of CYP27B1 in the development of MS is unclear.

6.284 Data are lacking on the relationship between vitamin D and type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis and SLE.

Infectious diseases

6.285 A possible role of vitamin D in modulating the immune response has been suggested by the presence of VDRs and 1α-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 TLR activation of human macrophages upregulated expression of the VDR and 1α-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and restricting growth 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.

6.286 Support for an immunomodulatory role has also been suggested by ecological studies showing associations between seasonal variations in serum 25(OH)D concentration and incidence of various infectious diseases including respiratory infection (Grant, 2008) and influenza (Cannell et al., 2006).

6.287 Possible mechanisms for the role of vitamin D 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.

6.288 IOM Report: The IOM considered the effect of vitamin D on tuberculosis (TB), influenza and upper respiratory infections. It 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 (Tables 50-52, Annex 2)

6.289 There is some evidence to suggest that vitamin D supplementation can influence the risk of developing infectious disease; however, 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.

6.290 Another difficulty that potentially complicates interpretation of the data on vitamin D and infection is the reported decrease in serum 25(OH)D concentration during the acute phase response to inflammation that occurs with infection (Silva & Furlanetto, 2015) (see paragraph 4.10).

Tuberculosis

6.291 TB is an infection caused by the bacterium *Mycobacterium tuberculosis* which typically affects the lungs and is transmitted by inhalation of airborne particles. A person exposed to TB will not necessarily develop the disease. Instead, the infection can remain inactive for many years; this is known as latent TB infection (LTBI). If the host immune system becomes weakened the infection can develop into active TB and spread to the lungs or other parts of the body, with symptoms developing within a few weeks or months. The tuberculin skin test (TST) conversion is the standard method used to determine whether a person is infected with *M tuberculosis.*

RCTs

6.292 Most RCTs have tested whether vitamin D therapy improves TB outcomes and might be used as an adjunctive treatment. Only 1 feasibility trial on vitamin D supplementation for the prevention of active TB in those with a latent infection could be identified. Ganmaa et al. (2012) assessed effects of daily vitamin D supplementation (20 µg/800 IU) on resistance to TST conversion among healthy school children in Mongolia (n=120; age, 12-15y). At baseline, mean serum 25(OH)D concentration was 18 nmol/L; 16 children in the vitamin D group and 18 in the placebo group were TST positive (p=0.7). After 6 months of intervention, mean serum 25(OH)D concentration increased in the vitamin D supplementation group (50 nmol/L) and decreased in the placebo group (10 nmol/L). TSTs converted to positive in 5 (11%) children receiving vitamin D compared with 11 (27%) receiving placebo but this

difference was not significant (RR=0.41; 95% CI, 0.16-1.09; p=0.06). A full-scale trial by these investigators is ongoing and due to complete in 2019[75].

Observational studies

6.293 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 proposed as a possible cause of this seasonal pattern, another possible cause was increased crowding in winter.

6.294 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) comparing serum 25(OH)D concentration in tuberculosis patients (not yet commenced any treatment) and healthy controls (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 (IQR) of 16 (2.25-74.25) to 65.8 (43.8-130.5) nmol/L. A problem with case-control studies is that the association between low serum 25(OH)D concentration and TB could be due to reverse causality (i.e., low serum 25(OH)D concentrations are caused by the TB).

6.295 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 1.6 years (Arnedo-Pena et al., 2015a). 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).

6.296 Fewer studies have investigated LTBI. A prospective cohort study (Talat et al., 2010) examined serum 25(OH)D concentrations in household contacts (n=109) of patients with recently diagnosed TB. Blood samples were collected at baseline, and at 6, 12 and 24 months follow-up. Eight percent progressed to active disease during 4 years of follow-up. TB progression was significantly associated with serum 25(OH)D concentrations < 17.5 nmol/L compared to concentrations >17.5 nmol/L (p=0.02). Arnedo-Pena et al. (2011) examined the association between serum 25(OH)D concentration and LTBI prevalence and TST conversion in contacts of TB patients (n=202). 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). Another prospective cohort study (Arnedo-Pena et al., 2015b) examined the association between serum 25(OH)D concentration and TB infection conversion (TBIC) in contacts of pulmonary TB patients (n=198) in Spain. After 8-10 weeks, 18 presented with TBIC. The mean serum 25(OH)D concentration in the TIBC cases (51.7 nmol/L) was significantly lower (p=0.03) than that of non-cases (67.9 nmol/L) and an increase of 2.5 nmol/L decreased TBIC incidence by 6% (RR=0.94; 95% CI, 0.90-0.99; p=0.015). However, serum 25(OH)D concentrations were only measured on one occasion so seasonal variation

[75] NCT02276755: The goal of this clinical trial is to investigate the preventive role of vitamin D supplementation in school age children in a high transmission setting.

was not taken into consideration and potential effects of other factors such as poverty and crowding were not considered.

Genetic studies

6.297 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; Arnedo-Pena et al., 2011) 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%).

6.298 A subsequent meta-analysis on VDR polymorphisms (FokI, TaqI, ApaI 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 with the TaqI and ApaI polymorphisms. However there were no significant associations between any of the polymorphisms and TB among African or South American populations.

Acute respiratory tract infections

6.299 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. The vocal cords are classically regarded as the cut-off between URTIs and LRTIs.

6.300 URTIs include tonsillitis, laryngitis and the common cold. LRTIs include bronchitis and pneumonia. Influenza affects both the upper and lower respiratory tracts.

Intervention studies

6.301 A systematic review and meta-analysis included 11 RCTs (n=5660; mean age, 16y), however 4 of these were in patient groups (Bergman et al., 2013). Vitamin D supplementation significantly reduced the risk of RTI (OR=0.64; 95% CI, 0.49-0.84; p=0.001) but 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 trials with daily vitamin D supplementation (OR=0.51; 95% CI, 0.39-0.67) but not in those which administered vitamin D in bolus doses once per month or less (OR=0.86; 95% CI, 0.60-1.20). There was no effect of baseline serum 25(OH)D concentration on supplementation outcome.

6.302 Another meta-analysis of 7 RCTs (n=4827) in healthy populations (Mao & Huang, 2013), which included the same RCTs as the meta-analysis by Bergman et al. (2013) but excluded the 4 RCTs in patient groups, reported no difference in RTI risk between the supplemented and control groups (RR=0.98; 95% CI, 0.93-1.03; p=0.45). Vitamin D dosing regimen, age and length of follow-up did not affect risk and there was no evidence of publication bias.

6.303 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.

6.304 Out of 7 RCTs published subsequent to these meta-analyses, 5 reported that vitamin D supplementation did not reduce RTI risk (Rees et al., 2013; Goodall et al., 2014; Urashima et al., 2014; Dubnov-Raz et al., 2015; Simpson et al., 2015). One RCT (Martineau et al., 2015) reported that vitamin D supplementation did not influence time to first RTI but when URTIs and LRTIs were analysed separately, vitamin D supplementation was associated with increased risk of URTI (HR=1.48, 95% CI 1.02 to 2.16, p=0.039) and increased duration of URTI symptoms (p=0.005) but not with altered risk or duration of LRTI. In the remaining RCT (Grant et al., 2015), healthy pregnant women (n=260; from 27 weeks gestation to birth) and their infants (from birth to 6 months) were assigned to 1 of 3 mother/infant groups: daily placebo/placebo; 25/10 μg/d (1000/400 IU) or 50/20 μg/d (2000/800 IU) of vitamin D_3. Compared to the placebo group, the proportion of children making any RTI visits was smaller in the higher-dose (p=0.004) but not the lower-dose vitamin D group (p=0.17).

Observational studies

6.305 The systematic review by Jolliffe et al. (2013) (see paragraph 6.303), 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.

6.306 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=5.21; 95% CI, 1.91-14.27; p trend=0.0004) during the first 6 months of life.

6.307 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). 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.

6.308 A cohort study of children and adolescents (n=743; age, 7-13y) 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).

6.309 A prospective population based cohort study of adults in Finland (n=1421; age, 53-73y) 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).

6.310 A retrospective cohort study in Denver (Jovanovich et al., 2014) compared patients (n=132; age, 60y) 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 vs ≥ 37 nmol/L was associated with increased odds of CAP (OR=2.57; 1.08-6.08; p=0.03).

Summary - Infectious diseases

6.311 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.

6.312 Evidence on vitamin D and infection is inconsistent and mainly observational. RCTs do not generally show a beneficial effect of vitamin D supplementation on infectious disease risk.

6.313 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.

6.314 Out of 3 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, 1 reported no effect and 1 reported conflicting results. The majority of RCTs published since the meta-analyses also did not find that vitamin D supplementation reduced RTI risk. 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.

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

6.315 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).

6.316 IOM Report: 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.

Evidence considered (Tables 53-54, Annex 2)

Cognition and dementia

Intervention studies

6.317 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, 87y), participants were supplemented with vitamin D_2 (1250 µg/50,000 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 D_2 supplementation had no effect on cognition.

6.318 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; median age, 77.5y). All participants were supplemented daily with vitamin D_2 (25 µg/1000 IU) throughout the trial. After 8 weeks run-in, participants were randomised to receive placebo or a high dose vitamin D_2 supplement

(300 µg/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.

6.319 Rossom et al. (2012) conducted a *post hoc* analysis of cognition related outcomes of women (n=4143; mean age, 71y) 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 D_3 (10 µg/400 IU daily) and calcium carbonate (1000 mg/d) or placebo. During a mean follow-up of 7.8 years, vitamin D and calcium supplementation was found to have no effect on cognitive decline or incident dementia.

Observational studies

6.320 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). Four of the 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

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

RCTs

6.322 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 group[76]. 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 measure[77] 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).

6.323 Another systematic review (Li et al., 2014) identified 6 RCTs (n=1203) that had investigated the effects of vitamin D_3 supplementation (at doses ranging from 38 µg/1500 IU to 178 µg/7100 IU daily) compared with placebo on depression in adults; 5 RCTs included adults at risk of depression and 1 RCT included depressed patients. Baseline serum 25(OH)D ranged between 47 to 100 nmol/L and study

[76] 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 studied which included participants whose baseline 25(OH)D concentrations indicated 'sufficiency'.
[77] Beck Depression Inventory.

duration varied between 8 weeks and 3-5 years. No significant effect of vitamin D supplementation was found on post intervention depression scores. There was substantial heterogeneity between studies (I^2=77%). Subgroup analyses stratified by vitamin D dose, study location or sex also did not show any effect of vitamin D supplementation.

6.324 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

6.325 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 et al., 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.

6.326 A systematic review and meta-analysis of observational studies (Anglin et al., 2013) (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

6.327 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.

Schizophrenia

Intervention studies

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

Observational studies

6.329 Epidemiological studies have reported a tendency for people with schizophrenia to be born in winter (Kellett et al., 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).

6.330 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 et al., 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).

6.331 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 -15 nmol/L (95% CI, -27, -3 nmol/L) but between study heterogeneity was significant (I^2 =97.6). The overall prevalence of 25(OH)D concentration < 50 nmol/L in schizophrenic patients was 65% (95% CI, 46-84%); however between study heterogeneity was significant (I^2 =84.8). Meta-analysis of the odds ratios reported in studies indicated that individuals with serum 25(OH)D concentration < 50 vs > 50 nmol/L were more likely to have schizophrenia (OR=2.16; 95% CI, 1.32-3.56).

Summary - Neuropsychological functioning

6.332 RCTs demonstrate no significant effect of vitamin D supplementation on cognition or depression. Evidence linking vitamin D to cognition and depression is supported mainly by cross-sectional data which suggest 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.

6.333 Evidence relating vitamin D to autism is very limited and mainly ecological.

6.334 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 and > 50 nmol/L is associated with increased schizophrenia risk.

Oral health

6.335 Vitamin D can impact on oral health by interference in mineralisation of teeth during their development and by modifying the rate of progression of bone loss during periodontal disease, which may result in more rapid tooth loss in people with low serum 25(OH)D concentration.

6.336 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 (Kikuchi et al., 1988; Zambrano et al., 2003) and in hypophosphataemic vitamin D resistant rickets (Nishino et al., 1990; Seow et al., 1995; Goodman et al., 1998; Murayama 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. These changes in the structure of enamel and dentine occur during tooth development from intra-uterine development up to around 18y of age. Any change in serum 25(OH)D concentration after the age of 18y will not affect the structure of the teeth.

6.337 In children with rickets, there is also increased susceptibility to periodontal disease or periodontitis, an inflammatory disease of the attachment apparatus of the tooth to the jaws. The supporting structures make up the periodontium and comprise the gingiva (or gum), the ligament that attaches the tooth to the bone and the alveolar bone that supports the tooth (Margvelashvili et al., 2014).

6.338 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 end stage of this pattern of disease progression is that the tooth becomes detached from the bone and is lost to function. This process is often accelerated by a dentist extracting the tooth when it becomes loose and sensitive. There is some evidence suggesting beneficial effects of anti-inflammatory agents in preventing periodontal bone loss in active teeth (Chapple et al., 2012). It is possible, therefore, that vitamin D could affect periodontal disease by modifying the rate of progression of bone loss or through anti-inflammatory effects.

6.339 IOM Report: The IOM did not consider vitamin D in relation to oral health.

Evidence considered

RCTs

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

Observational studies

6.341 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 ≥ 20y) 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).

6.342 A prospective cohort study in Germany, with 5 years follow-up (n=1904; age, 20-79y), 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

6.343 It has been suggested that associations between vitamin D and periodontal disease progression may be independent of it 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 (Hennig et al., 1999; Yoshihara et al., 2001; Sun et al., 2002; Brett et al., 2005; Park et al., 2006; Meng et al., 2007; Deng et al., 2011); as well as with chronic adult disease (Tachi et al., 2003; de Brito Junior et al., 2004; Martelli et al., 2011). 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

6.344 Low serum 25(OH)D concentration during tooth development results in alterations in the structure of tooth enamel and dentine.

6.345 Evidence from RCTs on effects of vitamin D supplementation on periodontal disease is lacking.

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

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

Age-related macular degeneration

6.348 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 drusen[78]. 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).

6.349 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.

6.350 IOM Report: The IOM report did not consider age-related macular degeneration.

Observational studies

6.351 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=7752; age, ≥ 40y) (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 in serum 25(OH)D concentration between those with (n=1045; mean age, 78y) and without (n=8124; mean age, 76y) AMD.

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

6.352 Millen et al. (2011) investigated the relationship between serum 25(OH)D concentration and prevalence of early AMD in postmenopausal women (n=1313; age, 50-79y) 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 lowest[79] quintile) was significantly associated with a lower risk of early AMD in women < 75y but an increased risk in older women; however this association was no longer significant after further adjustment for BMI and physical activity.

6.353 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=2525) 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 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

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

6.355 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

6.356 Intervention studies do not suggest beneficial effects of vitamin D supplementation during pregnancy on maternal reproductive outcomes. The observational evidence is mixed.

6.357 The contribution made by vitamin D supplementation during pregnancy to the later serum 25(OH)D concentration of the unsupplemented, exclusively breastfed baby is unclear. Maternal vitamin D supplementation during pregnancy has beneficial effects in reducing the risk of neonatal hypocalcaemia but there is little evidence from intervention or observational studies to indicate any additional benefits for the baby.

6.358 The small number of available RCTs have not shown an effect of vitamin D supplementation on overall cancer risk. Observational evidence suggests an inverse association between serum 25(OH)D concentration and colorectal cancer risk. These studies do not provide compelling evidence of a protective effect of vitamin D on colorectal cancer risk because they might be confounded by other

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

factors that affect cancer risk. Observational studies on other cancers have not found an association with serum 25(OH)D concentration.

6.359 Observational data from population cohort studies indicate a protective effect of higher serum 25(OH)D concentration on risk of CVD and hypertension but this finding is not supported by results from intervention trials.

6.360 Evidence from intervention studies indicates that vitamin D supplementation 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.

6.361 There is a paucity of data on the effect of vitamin D supplementation on immune modulation. Evidence from observational studies is inconsistent and may also be confounded by other factors that affect autoimmune disease and allergic disorders. The data are insufficient to draw firm conclusions.

6.362 RCTs do not generally show a beneficial effect of vitamin D supplementation on infectious disease risk. Evidence on vitamin D and infectious disease risk is mainly observational and suggests an inverse association between serum 25(OH)D concentration and infectious disease risk. However, 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.

6.363 Data on vitamin D and neuropsychological functioning is mainly observational and insufficient to draw conclusions. RCTs show no significant effect of vitamin D supplementation on cognition or depression. Cross-sectional data suggest an association between lower 25(OH)D concentration and poor cognitive function but this 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. Evidence relating vitamin D to autism and schizophrenia is mainly ecological.

6.364 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.

6.365 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

6.366 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.

6.367 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.

6.368 The evidence on vitamin D and musculoskeletal health was considered to be suggestive of beneficial effects of vitamin D on:

- rickets in infants and children;
- osteomalacia in all adult age groups;
- fall risk in adults ≥ 50y;
- muscle strength and function in young people and adults.

6.369 Musculoskeletal health was therefore selected as the basis for setting the DRVs for vitamin D.

6.370 Since serum 25(OH)D concentration reflects exposure to vitamin D from both sunlight and diet, the next step in the process was to identify a range of serum 25(OH)D concentrations required to protect musculoskeletal health or, if this was not possible, a threshold serum 25(OH)D concentration below which the risk of poor musculoskeletal health is increased. The current threshold used to indicate increased risk of vitamin D deficiency is a serum 25(OH)D concentration < 25 nmol/L (DH, 1998). Concentrations below this are associated with increased risk of rickets and osteomalacia.

6.371 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 could be identified.

- *Rickets* - Evidence is mainly observational and it is not clear whether, in all the cases reported, the cause of the rickets was vitamin D deficiency or calcium deficiency. In the majority of studies considered, individual or mean serum 25(OH)D concentration was < 25 nmol/L in children with rickets.
- *Osteomalacia* - Based on the limited evidence (mainly case reports) individual serum 25(OH)D concentrations were < 20 nmol/L. 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* - Evidence from RCTs is mixed but, on balance, is suggestive of beneficial effects of vitamin D supplementation in reducing fall risk in adults ≥ 50y with mean baseline serum 25(OH)D concentrations over a range of values. There is also evidence for an adverse effect of a high annual dose (12,500 µg/500,000 IU) or high monthly dose (1500 µg/60,000 IU or a combination of 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3).
- *Muscle strength and function* - Overall, evidence from RCTs suggests that vitamin D supplementation may improve muscle function in adolescents, adults < 50y with a mean serum 25(OH)D concentration < 30 nmol/L and in adults ≥ 50y with mean baseline serum 25(OH)D concentrations over a range of values.

6.372 With the exception of case reports, the studies considered only provided mean or median serum 25(OH)D concentrations of participants. It was not possible, therefore, to establish a range of serum 25(OH)D concentrations associated with the selected musculoskeletal health outcomes.

6.373 There was wide variability in the mean and individual serum 25(OH)D concentrations associated with increased risk of rickets, osteomalacia and falls and with improvement in muscle strength and function together with many uncertainties in the data. A particular limitation in trying to identify a range/threshold serum 25(OH)D concentration associated with beneficial effects on musculoskeletal health was the use of predefined cut-offs (based on different criteria for deficiency) in many studies.

As a result, cut-offs for deficiency in these studies are very insecure and it is not possible to assess if there is a dose response relationship.

6.374 Although there are many uncertainties in the data, the evidence is suggestive, overall, of an increased risk of poor musculoskeletal health at serum 25(OH)D concentrations below ~20-30 nmol/L.

6.375 An additional complexity for interpretation of the data is the high inter-assay and inter-laboratory variation in serum 25(OH)D concentration measurements (see chapter 4). A range of assay methods was utilised to measure serum 25(OH)D concentration in the different studies on musculoskeletal health, which makes it difficult to compare serum 25(OH)D concentrations associated with increased risk across various studies. 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 for increased risk of vitamin D deficiency, of < 25 nmol/L (DH, 1998), is retained.

6.376 This threshold serum 25(OH)D concentration of < 25 nmol/L, is not diagnostic of disease but denotes the concentration below which risk of poor musculoskeletal health is increased at a population level. It could, therefore, be considered 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* in the UK should achieve or be above in terms of protecting their musculoskeletal health.

6.377 Since the data were insufficient or inadequate to ascertain whether the threshold serum 25(OH)D concentration associated with increased risk of poor musculoskeletal health differs during pregnancy and lactation, the *'population protective'* concentration of 25 nmol/L was extended to these groups.

6.378 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 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.

6.379 The vitamin D intake and the summer sunshine exposure required to achieve a serum 25(OH)D target concentration of ≥ 25 nmol/L are considered in chapter 9.

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

Vitamin D toxicity

7.1 Cutaneous synthesis of vitamin D is regulated so that prolonged sunshine exposure does not lead to excess production (see paragraph 2.10). Excessive vitamin D intakes have, however, been shown to have toxic effects (Vieth, 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.

7.2 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 prostate 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

7.3 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

7.4 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.

7.5 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 (1000 IU) 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

7.6 The IOM (2011) selected onset of hypercalcaemia and related toxicity as the basis for establishing a Tolerable Upper Intake Level (UL) for all age groups except infants (0-12m). 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.

7.7 A UL of 100 µg/d (4000 IU) was set for adults ≥ 19y of age. A UL of 25 µg/d (1000 IU) was set for infants 0-6m and 38 µg/d (1520 IU) for infants aged 6-12m. The UL for pregnant and lactating women was the same as that for adults.

7.8 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 daily vitamin D intakes below 250 µg/(10,000 IU).

Europe

7.9 In 2012, the European Food Safety Authority (EFSA) revised the Tolerable Upper Intake Levels (UL[80]) (EFSA, 2012) 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).

7.10 For adults, hypercalcaemia was selected as the indicator of toxicity and the UL was set at 100 µg/d (4000 IU), including for pregnant and lactating women. A UL of 100 µg/d (4000 IU/d) was also set for children and adults aged 11-17y 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 (2000 IU/d) was set for children aged 1-10y to take account of their smaller body size. For infants (0-12m), the previous UL of 25 µg/d (1000 IU/d), 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[81]

7.11 For the purposes of the current review, the Committee on toxicity of chemicals in food, consumer products and the environment (COT):

- reviewed the data on potential harmful effects of high vitamin D intakes (either regularly over prolonged periods or as single or occasional large doses);
- considered whether some groups of people might be particularly vulnerable to high intakes of vitamin D.

7.12 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.

7.13 The findings and conclusions of the COT are summarised below. The COT's full statement with its detailed considerations is available on its website: (http://cot.food.gov.uk/sites/default/files/VitaminDstatement.pdf).

Review of the evidence on adverse effects

Adults

Hypercalcaemia

7.14 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 serum 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

[80] Definition of UL equivalent to that used by the IOM.
[81] The COT is an independent scientific committee that provides advice to Government Departments and Agencies on matters concerning the toxicity of chemicals.

and hypercalciuria can follow. Hypercalciuria is defined as urinary calcium excretion > 250 mg/d in women and 275-300 mg/d in men.

7.15 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.

7.16 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. Trials varied in design and few administered vitamin D doses > 100 µg/d (4000 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 (Barger-Lux et al., 1998; Heaney et al., 2003) used one or more doses ≥ 100 µg/d (4000 IU/d), in the absence of calcium supplements, for ≥ 2 months.

7.17 Heaney et al. (2003) investigated the relationship between steady state vitamin D_3 intake and serum 25(OH)D concentration. Vitamin D_3 doses of 0, 25, 125 or 250 µg/d (0, 1000, 5000 or 10,000 IU/d) 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/d (5000 IU/d) achieved serum 25(OH)D concentrations of 100-150 nmol/L (but not exceeding 150 nmol/L) after 160 days of administration.

7.18 Barger-Lux et al. (1998) investigated the relationship between graded oral dosing with vitamin D_3 for 8 weeks and changes in serum 25(OH)D concentration in healthy young men (n=116; mean age, 28y). Doses of 25, 250 or 1250 µg/d (1000; 10,000; or 50,000 IU/d) resulted in mean increases in serum 25(OH)D concentration of 28.6, 146.1 and 643.0 nmol/L respectively above the mean baseline concentration (67 nmol/L). No statistically significant changes were detected in mean baseline serum calcium concentration (2.41 mmol/L).

Kidney stones

7.19 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. An RCT of postmenopausal women (n=36,282; mean age, 62y) (Jackson et al. (2006) reported an increased risk of kidney stones in women given a daily calcium supplement of 1000 mg plus 10 µg (400 IU) of vitamin D for up to 7 years compared to those who received placebo (HR=1.17; 95% CI, 1.02-1.34). However, total intakes of vitamin D in this study were below those associated with hypercalcaemia.

Fall and fractures

7.20 An RCT (Sanders et al., 2010) of women in Australia (n=2256; age ≥ 70y) reported an increased risk of fracture in the vitamin D_3 supplemented group (single annual dose of 12,500 µg/500,000 IU for 3-5

years) compared to the placebo group (IRR=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.

7.21 Another RCT (Bischoff-Ferrari et al., 2016) of community-dwelling adults (n=200; age ≥70y with a prior fall) randomised to receive a monthly dose of either 600 µg (24,000 IU) vitamin D_3, 1500 µg (60,000 IU) vitamin D_3, or 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3 for 12 months reported that the incidence of falls was significantly higher in the 1500 µg (60,000 IU) vitamin D_3 group (66.9%; 95% CI, 54.4-77.5%) and the 600 µg (24,000 IU) vitamin D_3 + 300 µg 25(OH)D_3 group (66.1%; 95% CI, 53.5-76.8%) compared with the 600 µg (24,000 IU) vitamin D_3 group (47.9%; 95% CI, 35.8-60.3%) (p=0.048).

7.22 Another study (Smith et al., 2007) reported a significant increase in non-vertebral fracture in women (but not men) given an annual intra-muscular injection of vitamin D_2 (7500 µg/300,000 IU). No effect was observed on the frequency of falls.

7.23 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) and that serum concentrations ≥ 75 nmol/L were associated with a higher risk of fracture in Asian women.

Cancer

7.24 Pancreatic cancer: Some observational studies have reported an association between vitamin D intakes/ serum 25(OH)D concentration and risk of pancreatic cancer, but the findings have not been consistent. Skinner et al. (2006) reported that higher vitamin D intakes were significantly associated with a reduced risk of pancreatic cancer while Stolzenberg-Solomon et al. (2006) reported a 3-fold increase in pancreatic cancer risk in 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, p trend = 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 to < 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).

7.25 Prostate cancer: 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 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

7.26 The IOM (2011) identified 6 cohort studies (Sambrook et al., 2004; Sambrook et al., 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-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. Semba et al. (2009) did not observe a U-shaped relationship but median serum 25(OH)D concentration in the highest exposure category was 64 nmol/L.

7.27 A meta-analysis of 14 prospective cohort studies (Zittermann et al., 2012)[77] reported a summary RR for mortality of 0.71 (95% CI, 0.50-0.91) for serum 25(OH)D concentration ≥ 75 vs < 50 nmol/L. 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

7.28 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 µg/d (4000 IU).

Infants and children

7.29 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/4000 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/250-1200 IU per day) was found to be substantially lower than in the 1950s (100 µg/4000 IU per 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).

7.30 Early studies (Jeans & Stearns, 1938) showed that excess vitamin D could reduce linear growth in infants but this was not observed at doses up to 54 µg/d (2160 IU/d) (Fomon et al., 1966). The absence of effect was supported by a large prospective study of Finnish children (n=10,060) supplemented with 50 µg/d (2000 IU/d) of vitamin D (Hypponen et al., 2011). Growth was also not affected in breast-fed children whose mothers were given 25 or 50 µg/d (1000 or 2000 IU/d) of vitamin D from birth (Ala-Houhala et al., 1986). Calcium concentration, in studies where it was measured, was unaffected by vitamin D supplementation.

7.31 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 (50,000 IU)/twice weekly for 6 weeks) but hypercalcaemia was not observed.

7.32 Fewer data are available for older children but hypercalcaemia was not observed in children (n=8; age, 10-17y) receiving 350 µg (14,000 IU) per week of vitamin D_3 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=179) received weekly doses of vitamin D_3 (35 or 350 µg/1400 or 14,000 IU) or placebo for 1 year.

Setting a UL

7.33 A UL is the maximum intake that can be consumed every day over a life time without appreciable risk to health. ULs apply to specified population groups but may not be protective of individuals within those groups who have identifiable medical disorders that that cause them to be unusually vulnerable to a particular substance. ULs can be established using either human or animal data on adverse effects and incorporate uncertainty factors as appropriate.

7.34 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 ULs for vitamin D. Evidence for other potential adverse effects, which might occur at lower exposures, was considered to be inconsistent.

Adults

7.35 Based on data from 2 studies relating to hypercalcaemia (Barger-Lux et al., 1998; Heaney et al., 2003) EFSA set a no-observed-adverse-effect-level (NOAEL) of 250 µg/d (10,000 IU/d). 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 UL for vitamin D of 100 µg/d (4000 IU/d), 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 IU/d) set by EFSA, and agreed that a UL of 100 µg/d (4000 IU/d) was appropriate for adults (≥ 18 y).

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

Pregnancy and lactation

7.37 Neither the IOM nor EFSA adjusted the UL to take account of pregnancy or lactation. The COT agreed that the UL of 100 µg/d (4000 IU/d) set for adults (≥ 8 y) was appropriate for pregnant and lactating women.

Infants and children

7.38 The ULs set by the IOM for infants aged 0-6 and 6-12m of 25 and 38 µg/d (1000 & 1520 IU/d) respectively were based on studies on growth (Jeans & Stearns, 1938; Fomon et al., 1966) and considerations about IIH. EFSA (2012) retained the previous UL of 25 µg/d (1000 IU/d) (set in 2003) for children aged 0-12m, taking particular account of the studies by Jeans & Stearns (1938), Fomon et al. (1966), and Hypponen et al. (2011). The COT agreed that the UL of 25 µg/d (1000 IU/d) of vitamin D set by EFSA, for infants aged 0-12m, was appropriate.

7.39 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/d (4000 IU/d) to 62.5 µg/d (2500 IU/d) for children aged 1-3y and 75 µg/d (3000 IU/d) for children aged 4-8 y. The ULs set for children and adolescents aged 9-18y 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 (2000 IU/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 UL of 100 µg/d (4000 IU/d) was therefore set for children aged 10-17 y. A UL of 50 µg/d (2000 IU/d) was set for children aged 1-10y to take account of their smaller body size.

7.40 COT agreed that the ULs for vitamin D set by EFSA, of 50 and 100 µg/d (2000 and 4000 IU/d) for children aged 1-10y and 11-17y respectively, were appropriate.

Groups in which the ULs may not be protective

7.41 The ULs 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

7.42 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 1-20m), however, reported that vitamin D_2 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.

7.43 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 at doses ≥ 15,000 µg (600,000 IU).

Summary & conclusions

7.44 Acute and chronic exposure to excess vitamin D intake can result in hypercalcaemia, demineralisation of bone, soft tissue calcification and renal damage. Hypercalcaemia is the most appropriate endpoint on which to base ULs for vitamin D since adverse effects that might occur at lower doses, through other mechanisms, have not been reliably established.

7.45 ULs for vitamin D recommended by EFSA, of 100 µg/d (4000 IU/d) for adults and children aged 11-17 y, 50 µg/d (2000 IU/d) for children aged 1-10 y, and 25 µg/d (1000 IU) for infants, are considered appropriate. The ULs do not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D make only a small contribution to total exposures at the ULs.

7.46 The ULs 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 predisposition (e.g., IIH).

7.47 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 indicates toxicity could occur in infants at doses ≥ 15,000 µg (600,000 IU).

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

8.1 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 (RP), a continuous survey of diet and nutrition in adults and children aged 18m upwards. The results presented here are based on a UK representative sample of 3,450 adults aged 19y and above and 3,378 children aged 1½-18y 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 above (Finch et al., 1998). Representative data collected from boosted samples for Scotland (n=867 adults & 828 children), Wales (n=461 adults & 391 children) and Northern Ireland (n=470 adults & 512 children) were obtained from the NDNS RP Scotland (2008/09-2011/12)[82], the NDNS RP Wales (2009/10-2012/13)[83] and the NDNS RP Northern Ireland (2008/09-2011/12)[84] reports respectively.

8.2 Data on low income populations in the UK (aged 2y and above) from 2003-2005 were obtained from the Low Income Diet and Nutrition Survey (LIDNS) (Nelson et al., 2007a; Nelson et al., 2007b).

8.3 Data on infants and young children (aged 4-18m) were obtained from the 2011 UK Diet and Nutrition Survey of Infants and Young Children (DNSIYC) (Lennox et al., 2013).

8.4 Additional data were obtained from the Health Survey for England (HSE) 2005[85] and 2010[86] and the Scottish Health Survey (SHS) (FSA Scotland[87], 2013).

8.5 Nationally representative data are not available for pregnant or breastfeeding women. Data presented for pregnant women were obtained from UK based cohort studies.

Assessment of vitamin D intakes

8.6 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.

8.7 In the NDNS, 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 (DLW[88]) technique. The discrepancy is probably due to a combination of under-reporting of actual consumption and changing the diet during the recording period. It is not possible to extrapolate these estimates of under-reporting of energy intake to individual foods or nutrients nor to correct or adjust intake estimates to take account of under-reporting.

[82] NDNS RP: results from Years 1- 4 (combined) for Scotland (2008/09-2011/12) http://www.food.gov.uk/sites/default/files/ndns-scotland-full-report.pdf
[83] NDNS RP: results from Years 2-5 (combined) for Wales (2009/10-2012/13) http://gov.wales/statistics-and-research/national-diet-nutrition-survey-rolling-programme/?lang=en
[84] NDNS RP: results from Years 1- 4 (combined) for Northern Ireland (2008/09-2011/12) http://www.food.gov.uk/sites/default/files/ndns-ni-full-report.pdf
[85] National Centre for Social Research, University College London. Department of Epidemiology and Public Health. (2011). *Health Survey for England, 2005*. [data collection]. *3rd Edition.* UK Data Service. SN: 5675, http://dx.doi.org/10.5255/UKDA-SN-5675-1.
[86] NatCen Social Research, Royal Free and University College Medical School. Department of Epidemiology and Public Health. (2015).*Health Survey for England, 2010*. [data collection]. *3rd Edition.* UK Data Service. SN: 6986, http://dx.doi.org/10.5255/UKDA-SN-6986-3.
[87] Food Standards Agency.
[88] The DLW technique is included as an objective biomarker to validate energy intakes estimated from reported food consumption.

8.8 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.

8.9 There is no evidence of under-reporting for vitamin D specifically.

Dietary sources of vitamin D

8.10 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 infants (aged 4-18m).

8.11 In the NDNS, meat and meat products were the major contributors to vitamin D intake for all age groups, except children aged 1.5-3y, providing 23-35% of intake. Milk and milk products were the major contributors to vitamin D intake for children aged 1.5-3y, 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).

8.12 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%).

8.13 In LIDNS the main sources of vitamin D were meat and meat products (adults, 30%; children, 32%) and fat spreads (26% for adults and children). Cereals and cereal products made a greater contribution to vitamin D intakes of children than adolescents (about 20% in children aged 2-10y compared with around 15% in adolescents ages 11-18y).

8.14 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 (Finglas et al., 2015)

Food	Mean vitamin D content per 100g		Vitamin D content of typical portion size	
	µg	IU	µg	IU
FISH				
Herring *(grilled)*	16.1	644	19.2	768
Salmon *(farmed, grilled)*	7.8	312	8.3	332
Salmon *(farmed, steamed)*	9.3	372	9.9	396
Salmon *(pink, canned in brine, drained)*	13.6	544	14.4	576
Salmon *(cold & hot smoked)*	8.9-11	356-440	5.0-6.2	200-248
Mackerel *(grilled)*	8.5	340	13.6	544
Mackerel *(smoked)*	8.2	328	12.3	492
Sardines *(grilled)*	5.1	204	4.4	176
Sardines *(canned in brine, drained)*	3.3	132	1.5	60
Tuna *(baked)*	3.1	124	4.7	188
Tuna *(canned in brine, drained)*	1.1	44	0.9	36
EGGS				
Eggs *(whole, boiled)*	3.2	128	1.7	68
Eggs *(yolk, boiled)*	12.6	504	2.3	92
MEAT				
Liver *(lamb, fried)*	0.9	36	0.9	36
Liver *(calf, fried)*	0.3	12	0.3	12
Beef *(rump steak, fried)*	0.7	28	1	40
FORTIFIED FOODS				
Bran flakes	4.2	168	1.3	52
Cornflakes	4.2	168	1.3	52
Rice cereal	4.2	168	1.3	52
Fat spreads *(reduced fat 62-75% polyunsaturated)*	7.5	300	0.75	30

Vitamin D intakes in the UK (Tables 1-18, Annex 3)

8.15 Data on mean vitamin D intakes in Scotland, Wales and Northern Ireland are generally similar to those for the overall UK population and are therefore not detailed in this section but are provided in Annex 3 (Tables 10-12).

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

Infants and young children (4-18 months)

8.17 Mean intakes of vitamin D from food sources was higher in non breastfed infants compared to breast fed infants (excluding intake from breast milk, as the vitamin D content of breast milk is unknown).

8.18 For non breast fed infants, mean daily intakes of vitamin D were 9.8 µg/392 IU (4-6m), 8.7 µg/348 IU (7-9m), 7.5 µg/300 IU (10-11m) and 3.5 µg/140 IU (12-18m). For breastfed infants (excluding breast milk) mean daily intakes were 3 µg/120 IU (4-6m), 3.2 µg/128 IU (7-9m), 2.7 µg/108 IU (10-11m) and 1.8 µg/72 IU (12-18m).

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

Children (1.5-10y)

8.20 For children aged 1.5-3y, mean daily intake of vitamin D was 1.9 µg (76 IU) from food sources and 2.3 µg (92 IU) from all sources (including supplements). Mean vitamin D intake from all sources was 32% of the RNI for children aged 1.5-3y.

8.21 The mean daily vitamin D intake for children aged 4-10y was 2 µg (80 IU) from dietary sources and 2.7 µg (108 IU) from all sources (including supplements).

8.22 In the LIDNS, the mean daily vitamin D intake (from food sources only) for boys and girls aged 2-10y was 2 and 1.7 µg (80 and 68 IU) respectively. Mean daily intakes were 22% of the RNI for children aged 2-10y[89].

Adolescents (11-18y)

8.23 The mean daily vitamin D intake was 2.1 µg (84 IU) from dietary sources and 2.4 µg (96 IU) from all sources (including supplements).

8.24 In the LIDNS, the mean daily vitamin D intake (from food sources only) for boys and girls was 2.4 and 2 µg (96 and 80 IU) respectively.

Adults 19-64y

8.25 The mean daily intake of vitamin D from dietary sources was 2.8 µg (112 IU). Vitamin D supplements increased mean daily intakes to 3.9 µg (156 IU) in men and 3.4 µg (136 IU) in women.

8.26 In the LIDNS, mean daily vitamin D intakes (from food sources only) for men aged 19-49 and 50-64y were 3.0 and 3.7 µg (120 and 148 IU) respectively; for women aged 19-34, 35-49 and 50-64y mean daily intakes were 2.2, 2.5, 2.8 µg (88, 100, 112 IU) respectively.

Adults 65y and above

8.27 The mean daily vitamin D intake from dietary sources was 3.3 µg (132 IU). Dietary supplements containing vitamin D made the largest contribution to intakes in this age group, increasing daily mean intakes to 5.1 µg (204 IU) in men and 5.2 µg (208 IU) in women. The mean intake was 51% of the RNI.

8.28 For institutionalised adults, mean daily vitamin D intake of men was 3.9 µg/156 IU (39% of RNI) from all sources and 3.8 µg/152 IU (38% of RNI) from food sources; for women, mean daily intake was 3.4 µg/136 IU (34% of RNI) from all sources and 3.3 µg/132 IU (33% of RNI) from food sources.

8.29 In the LIDNS, mean daily vitamin D intakes (from food sources only) were 3.4 µg (136 IU) for men and 2.6 µg (104 IU) for women. Mean daily intake of vitamin D was 34% of the RNI for men and 26% for women.

[89] No RNIs were set for children aged 4-10 years.

Serum/plasma 25(OH)D concentrations in the UK (Tables 19-36, Annex 3)

Assessment of serum/plasma 25(OH)D concentration

8.30 Serum/plasma 25(OH)D concentration reflects the availability of vitamin D in the body from both dietary and endogenous sources. In the UK a serum/plasma 25(OH)D concentration < 25 nmol/L is currently used to indicate risk of vitamin D deficiency (DH, 1998).

8.31 The main problems associated with the methods used for measuring serum/plasma 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 (further details in chapter 4).

8.32 Blood collection within the NDNS rolling programme, LIDNS, HSE and SHS is spread evenly across the year so the values presented are year round averages. In the DNSIYC blood samples were collected between February and August. Plasma samples were analysed in the NDNS and SHS while serum samples were analysed in the LIDNS, HSE and DNSIYC.

Infants (4-18m)

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

Children (1.5-3y)

8.34 Mean plasma 25(OH)D concentration in children aged 1.5-3y was 58 nmol/L; 7.5% had concentrations < 25 nmol/L, however this is based on a small sample (n=42).

Children and adolescents (4-18y)

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

8.36 Mean plasma 25(OH)D concentrations for boys and girls aged 11-18 y were 44.9 nmol/L and 41.1 nmol/L respectively; 19.7% of boys and 24.4% of girls had a plasma 25(OH)D concentration < 25 nmol/L.

8.37 In LIDNS, mean serum 25(OH)D concentration was 43.5 nmol/L for boys and 39.6 nmol/L girls aged 11-18y; 8% of boys and 23% of girls had a plasma 25(OH)D concentration < 25 nmol/L.

Adults 19-64y

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

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

Adults 65y and above

8.40 In the NDNS, mean plasma 25(OH)D concentration was 47 nmol/L in men and 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.

8.41 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.

8.42 In the HSE 2005 mean serum 25(OH)D concentration was 53 nmol/L for men and 48 nmol/L for women.

8.43 In the LIDNS, mean serum 25(OH)D concentration) was 52.8 nmol/L in men and 44.2 nmol/L in women; 14% of men and women had a serum 25(OH)D concentration < 25 nmol/L.

Serum/plasma 25(OH)D concentration by season

8.44 For all age groups in the NDNS, mean plasma 25(OH)D concentrations were lowest during the winter months (January-March) and highest in the summer months (July-September). For children (4-10y) mean plasma 25(OH)D concentration was 37 nmol/L in the winter months and 66 nmol/L in the summer months. For older children and adolescents (11-18y) 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 19-64y, the mean 25(OH)D concentration was 34.8 nmol/L in the winter and 57.5 nmol/L in the summer. For adults aged ≥ 65y the mean plasma 25(OH)D concentration was 40.5 nmol/L in winter and 50.5 nmol/L in the summer.

8.45 The proportion of the population with a plasma 25(OH)D concentration < 25 nmol/L in the winter months was 4-10y, 31%; 11-18y, 40%; 19-64y, 39%; ≥ 65y, 29%. The proportion with plasma 25(OH)D concentration < 25 nmol/L in the summer months was: 4-10y, 2%; 11-18y, 13%; 19-64y, 8%; and ≥ 65y, 4%.

8.46 Serum 25(OH)D concentration was also measured by season in the HSE (2010) and the SHS. For ages ≥ 16y, 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 serum 25(OH)D concentration was 33.1 nmol/L in England 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 serum 25(OH)D concentration < 25 nmol/L was 42% in winter and 7% in summer; in Scotland, the proportion with plasma 25(OH)D concentration < 25 nmol/L was 54% in winter and 17% in summer.

8.47 A cohort study of South Asian women (n=35) living in Southern England (Darling et al., 2013) found that 81% and 53% had a plasma 25(OH)D concentration < 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 < 25 nmol/L was 49% in winter and 29% in summer.

Serum/plasma 25(OH)D concentration by region

8.48 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.

8.49 Data from NDNS Scotland, NDNS Wales or NDNS Northern Ireland were not split by season due to small sample sizes.

8.50 In Scotland, the mean plasma 25(OH)D concentration was 47 nmol/L for children aged 4-10y, 37 nmol/L for ages 11-18y, 40 nmol/L for adults aged 19-64y and 42 nmol/L for adults aged ≥ 65y. The proportion of these age groups with a plasma 25(OH)D concentration < 25 nmol/L was: 9%, 4-10y; 26%, 11-18y, 33%, 19-64y; and 29%, ≥ 65y.

8.51 In Northern Ireland, the mean plasma 25(OH)D concentration was 39 nmol/L for ages 11-18y, 33 nmol/L for ages 19-64y and 45 nmol/L for≥ 65y. The proportions with a plasma 25(OH)D concentration < 25 nmol/L was: 30%, 11-18y, 34%, 19-64y; and 19%, ≥ 65y.

8.52 In Wales, the mean plasma 25(OH)D concentration was 43 nmol/L for children aged 11-18y, 51 nmol/L for men and 43 nmol/L for women aged 19-64y and 43 nmol/L for adults aged ≥ 65y. The proportion with a plasma 25(OH)D concentration < 25 nmol/L was: 23%, 11-18y; 20% of men & 16% of women, 19-64y; and 17%, ≥ 65y.

Serum 25(OH)D concentration during pregnancy

8.53 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). The mean serum concentration was 38 nmol/L in summer (July-September) and autumn (October-December), 26 nmol/L in winter (January-March) and 32 nmol/L in spring (April-June). The percentage with a serum 25(OH)D concentration < 25 nmol/L ranged from 29% in the summer to 49% in the winter.

8.54 In the Southampton Women's Survey, the median serum 25(OH)D concentration of pregnant women (n=977; predominantly white) measured at 34 weeks gestation was 62 nmol/L; 35% had a serum 25(OH)D concentration < 50 nmol/L (Crozier et al., 2012).

8.55 Blood samples (taken throughout the year) were also available for pregnant women (n=3,960; predominantly white) taking part in the ALSPAC[90] 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% had a serum 25(OH)D concentration < 27.5 nmol/L.

8.56 A study in Aberdeen (Haggarty et al., 2013) measured plasma 25(OH)D concentrations of pregnant women (n=1205; mean age at delivery, 31.5y; predominantly white) at 19 weeks gestation. Mean concentrations were 53 nmol/L in summer (June-August), 34 nmol/L in autumn (September-November) and winter (December-January) and 40 nmol/L in spring (March-May). The proportions with plasma 25(OH)D concentration < 25 nmol/L were 25% in summer, 43% in spring, 60% in autumn and 76% in winter.

[90] Avon longitudinal survey of parents and children.

Serum 25(OH)D concentration by ethnicity

8.57 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 (≥ 16y): 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.

8.58 The 1996 Asian Infant Feeding Survey (Lawson & Thomas, 1999) measured serum 25(OH)D concentration in Asian children (n=618; age, 2y) in England. The mean serum 25(OH)D concentration in Bangladeshi, Pakistani and Indian infants was 42.1, 36.2 and 42.2 nmol/L respectively. Twenty percent of Bangladeshi infants, 34% of Pakistani infants and 25% of Indian infants had a serum 25(OH)D concentration < 25 nmol/L.

8.59 A prospective cohort study (Darling et al., 2013) measured serum 25(OH)D concentration in South Asian (n=35; mean age, 38y) and white women (n=105; mean age, 34y) living in Southern England (Surrey) throughout the year (2006-2007). Overall, the South Asian women had lower serum 25(OH)D concentrations than white women and a higher percentage had a serum 25(OH)D concentration < 25 nmol/L. In autumn 2006 and spring 2007, the percentage of South Asian women with a serum 25(OH)D concentration < 25 nmol/L was 79% and 75% respectively compared to 4% and 5% of white women. The lowest mean 25(OH)D concentration was recorded in the winter months (19.7 nmol/L for South Asian women; 44.5 nmol/l for white women).

8.60 In Scotland, Haggarty et al. (2013) reported that plasma 25(OH)D concentrations in a cohort of pregnant women (n=1205) were significantly lower (difference of 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

8.61 The HSE analysed serum 25(OH)D concentrations by BMI (kg/m^2). For adults, mean serum 25(OH)D concentrations 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).

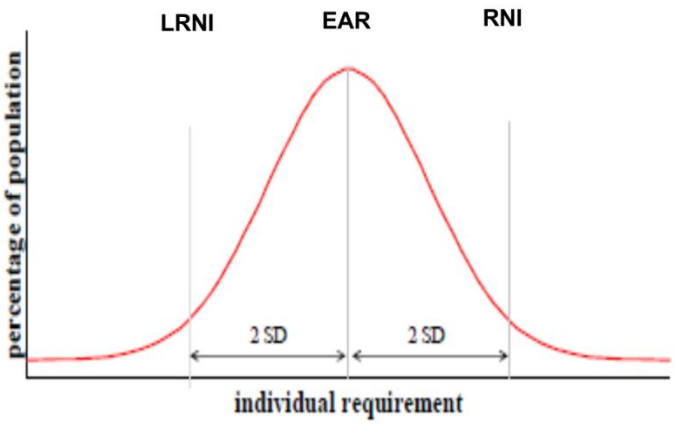
8.62 Haggarty et al. (2013) found no association between body weight or BMI and plasma 25(OH)D concentration during pregnancy.

9. Review of DRVs for vitamin D

Brief overview of DRVs

9.1 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 7). 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 intakes which will meet the needs of some individuals in the group who have low requirements (approximately 2.5%). Intakes below the LRNI are almost certainly inadequate for most individuals.

FIGURE 7: Dietary Reference Values – definitions



9.2 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 (i.e., those aged 4-64y). Instead, the assumption was that vitamin D synthesised cutaneously in summer through sunlight exposure would be sufficient to maintain serum 25(OH)D concentrations above 25 nmol/L[91] in the following winter months. An RNI only was established for the following population age groups: 0-6m (8.5 µg/340 IU per day); 7m-3y (7.0 µg/280 IU per day); and 65y and above (10 µg/400 IU per day). An RNI (10 µg/400 IU per day) was also set for pregnant and lactating women and Asian women and children.

[91] 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

9.3 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 3.35-3.38). 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 outcomes and serum 25(OH)D concentration to use as the basis for setting DRVs

9.4 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)
• Rickets (*infants and children*) • Osteomalacia (*all adult age groups*) • BMC/BMD (*all life stages*) • Stress fracture risk (*adults < 50y, mainly athletes and military personnel*) • Fracture prevention (*adults ≥ 50y*) • Fall risk (*adults ≥ 50y*) • Muscle function and power (*adults < and ≥ 50y)*
Non-musculoskeletal health outcomes
• Pregnancy & lactation – non-musculoskeletal health outcomes • Cancers • CVD & hypertension • All-cause mortality • Immune modulation • Infectious diseases • Neuropsychological functioning • Oral health • Age-related macular degeneration

9.5 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 and in improving muscle strength and function in adolescents and adults (< and ≥ 50y). 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**.**

9.6 As described 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 evidence is suggestive of an increased risk of poor

musculoskeletal health at serum 25(OH)D concentrations below 25 nmol/L (based on studies of rickets in children, osteomalacia in adults, falls in adults ≥ 50y and muscle strength and function in adolescents and adults).

9.7 It is important to recognise that a serum 25(OH)D concentration of 25 nmol/L is not a clinical threshold diagnostic of disease but 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. It is not a *mean* target serum 25(OH)D concentration for a particular life-stage group but rather the concentration that the majority (97.5%) of ***individuals*** in the UK should achieve or be above to protect their musculoskeletal health.

9.8 A serum 25(OH)D concentration of 25 nmol/L was, therefore, used 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 in 97.5% of the population.

9.9 Data from the NDNS show that the majority of UK population achieves a plasma 25(OH)D concentration > 25 nmol/L during the summer months and this is probably from skin synthesis of vitamin D in response to sun exposure. However, sun exposure is notably reduced as a contributor to plasma 25(OH)D concentrations of the UK population in the winter months and skin synthesis of vitamin D in the winter months is negligible in the UK population. Data from the NDNS and other studies indicate that between 29 and 54% of various population groups in the UK have a serum or plasma 25(OH)D concentration < 25 nmol/L in the winter (see paragraphs 8.45-8.46).

9.10 Current recommendations (DH, 1991) assume that enough vitamin D is synthesised cutaneously 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 previously set for individuals aged 4-64y. However, it is clear that summer synthesis of vitamin D does not ensure a winter serum 25(OH)D concentration ≥ 25 nmol/L in a substantial proportion of the UK population. In addition, although most people are able to synthesise vitamin D in the summer and achieve serum 25(OH)D concentrations ≥ 25 nmol/L naturally from sun exposure, there is a proportion (7-53%) of some population groups in the UK with serum 25(OH)D concentration < 25 nmol/L during the summer (see paragraphs 8.45-8.47). Since it is not possible to identify these individuals, and because the RNI is intended to cover the needs of 97.5% of the population, it is proposed that the RNI should apply throughout the year as a precautionary measure.

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

9.11 The IOM reviewed the data on vitamin D and a range of health outcomes to assess their suitability as a basis for developing 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 RDA[92]); 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.

[92] 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.

9.12 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 outcomes was not considered sufficient to inform 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.

9.13 Using the 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 considered 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.

9.14 The considerations in 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.

9.15 In the current review, the data were not considered sufficient to establish a distribution of serum 25(OH)D concentrations that would be necessary for estimating 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 poor musculoskeletal health is 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 that used by the IOM in selecting a serum 25(OH)D concentration of 50 nmol/L as being consistent with an RDA-type value (i.e., covering the needs of 97.5% of the population).

Modelling exercise

9.16 Two modelling options for attaining year-round serum 25(OH)D concentration ≥ 25 nmol/L were investigated:

- the summer sunshine exposure required to maintain serum concentration ≥ 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 ≥ 25 nmol/L.

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

9.17 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 but the small amount of UVB in winter sunlight means that vitamin D synthesis is negligible from at least October through early March (Webb et al., 1988) and serum 25(OH)D concentrations decline throughout the winter.

9.18 A UK study (Kazantzidis et al., 2015)[93] 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 ≥ 25 nmol/L in the winter.

9.19 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 (2003-2012) were used to calculate UV wavelengths arriving at the Earth's surface which were then weighted with the different action spectra[94] (for previtamin 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.

9.20 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 studies 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.

9.21 The shortest and safest exposure regimen was used in the modelling exercise. 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.

9.22 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 > 25 nmol/L in winter if all individuals achieved a serum 25(OH)D concentration ≥ 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.

9.23 Skin exposure area and duration to achieve serum 25(OH)D concentration ≥ 80.5 nmol/L were also modelled. It was estimated that with 35% skin area exposed (equivalent to wearing modest

[93] This study was specifically commissioned by the Department of Health to inform SACN's review of the DRVs for vitamin D.
[94] Relative efficacy of different wavelengths in causing an effect.

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 (≥ 80.5 nmol/L) 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.

9.24 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 regimen 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.

9.25 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 hands and face.

9.26 There are a number of limitations in these estimations since the two main inputs for the model, weather and 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. 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, incident UV radiation is significantly reduced. Another assumption is that skin on all parts of the body synthesises vitamin D at the same rate.

9.27 An important limitation of the modelling 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 serum 25(OH)D concentration ≥ 25 nmol/L

9.28 As outlined in the previous section, making recommendations on sunlight exposure as a means of achieving and maintaining serum 25(OH)D concentration ≥ 25 nmol/L for 97.5% of the population is problematic. Taking account of sunlight exposure in setting the RNI (i.e., dietary intake) for vitamin D is also complicated by the number of factors that impact on cutaneous production of vitamin D.

9.29 The process of translating the target serum 25(OH)D concentration of ≥ 25 nmol/L into an RNI was therefore based on RCTs 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 (7-53%) 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 ensure protection of 97.5% of the population.

9.30 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):

a. meta-regression approach;

b. approach using data from individual vitamin D RCTs.

(a) Meta-regression approach

9.31 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 (Nordic Council of Ministers, 2014).

9.32 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 (from foods and supplements). 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 et al., 2010; Carter, 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.

9.33 Another important limitation with the meta-regression model, 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 concentration 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 a higher threshold serum 25(OH)D concentration (50 nmol/L) to use as the basis for their recommendations.

(b) Approach using data from individual vitamin D RCTs

9.34 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.

9.35 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 (Cashman et al., 2008); adults aged ≥ 64y (Cashman et al., 2009); and adolescent girls aged 11 y (Cashman et al., 2011b). 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 to maintain serum 25(OH)D concentrations ranging from 25 to 80 nmol/L, during winter.

9.36 Data from two of these RCTs[95] (Cashman et al., 2008; Cashman et al., 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 [55-57ºN] than in London and the South East [51ºN]). The RCT in adolescent girls (Cashman et al., 2011b) 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 RCTs was specifically selected to provide a range 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 DACH[96] country recommendations for vitamin D but using 50 nmol/L as their basis (German Nutrition Society, 2012).

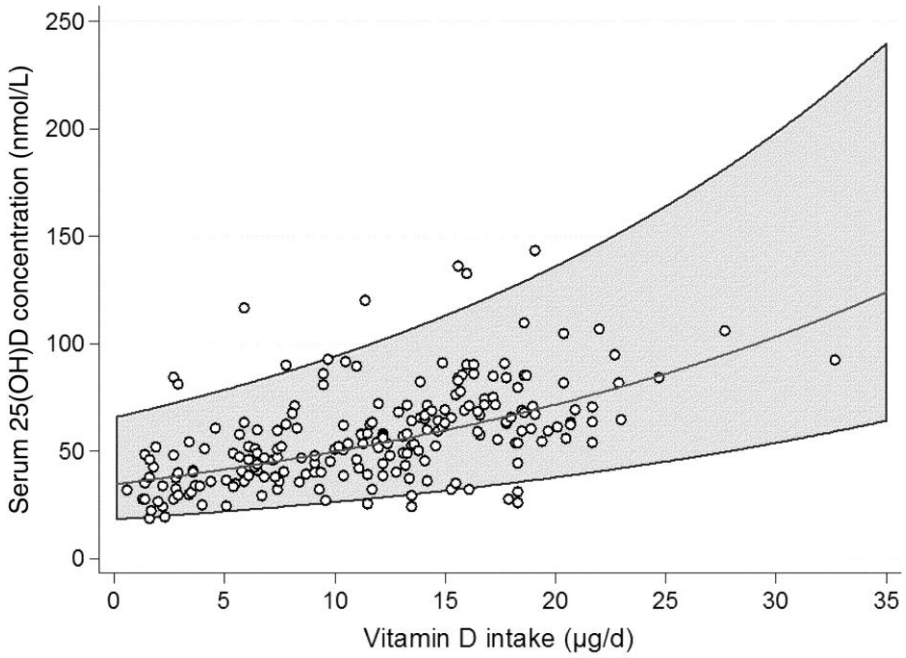
Further considerations in setting DRVs for vitamin D using the RCTs data

9.37 The RNI is notionally an intake of a nutrient that is two SDs above the EAR 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, in 97.5% of the population (i.e., the RNI). This direct estimation avoids the requirement to add two SDs to the EAR, which is an approximation[97].

9.38 Full details of the regression and mathematical modelling used to derive these intake estimates are provided in the three publications (Cashman et al., 2008; Cashman et al., 2009; Cashman et al., 2011b) 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[98] 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 8 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 were estimated. In all three age-groups, results were verified using robust regression models that minimised the effect of outliers and heteroscedasticity[99].

[95] 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.
[96] [D-A-CH arises from initial letters of the common country identification for the countries Germany (D), Austria (A) and Switzerland (CH)].
[97] 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.
[98] Quantile-Quantile plot is a graphical method for comparing two probability distributions.
[99] Occurs when variance around the regression line is unequal across the range of values of the predictor variable.

FIGURE 8: Relation between serum 25(OH)D concentrations (in late winter 2007) & total vitamin D intake (diet & supplements) in 20-40 year-old healthy persons (n = 215) living at northerly latitudes (51 & 55 °N) (Cashman et al., 2008)



9.39 Using data from individual RCTs for adults aged 20-40y and ≥ 64y and teenage girls (Cashman et al., 2008; Cashman et al., 2009; Cashman et al., 2011b), it is estimated that a vitamin D intake of 8.3-8.7 µg/d (332-348 IU) would be needed to maintain a 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 the 50th (EAR), 90th and 95th percentiles are also presented.

9.40 The estimated intakes are derived from serum 25(OH)D concentration data measured by immunoassay for adults (20-40 and ≥ 64y) and by HPLC for adolescent girls (11y). A comparison of serum 25(OH)D concentrations measured by immunoassay and standardized LC-MS/MS[100] 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; Cashman et al., 2009) indicate that a vitamin D intake of about 9 µg/d (360 IU) 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 found that about 12 µg/d (480 IU) is needed to achieve and maintain winter serum 25(OH)D concentration ≥ 25 nmol/L in 97.5% of adults.

9.41 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 that risk of musculoskeletal health outcomes is increased at serum 25(OH)D concentrations < 25 nmol/L). Since estimates of the daily vitamin D intake required to maintain serum 25(OH)D concentration ≥ 25 nmol/L in winter by 97.5% of the population differ

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

according to whether immunoassay (~9 µg/360 IU) or LC-MS/MS analysis (12 µg/480 IU) is used, an RNI of 10 µg/d (400 IU) was set between these two estimates.

RNIs for vitamin D by life-stage

9.42 The data used to estimate the vitamin D intake required to achieve a serum 25(OH)D concentration ≥ 25 nmol/L were drawn from individual RCTs in adults aged 20-40y, adults aged ≥ 64y and adolescent girls aged 11y. Dose-response data were not available to allow direct determination of the vitamin D intake needed to achieve a serum 25(OH)D concentration ≥ 25 nmol/L in infants and children aged 0-10y 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 (Madsen et al., 2013) examining the effects of vitamin D fortified milk and bread on serum 25(OH)D concentrations of children and adults (n=782), reported no difference in treatment effects by age group (4-10, 11-17, 18-40 and 41-60y). This suggests that data from the RCTs in adults (20-40y and ≥ 64y) and adolescent girls (aged 11y) can be extrapolated to younger age groups.

9.43 The RNI estimates for vitamin D assume that calcium intakes are adequate. However, findings from an RCT (Cashman et al., 2014a) indicate that calcium intake does not modify the requirement for vitamin D in healthy adults with intakes ranging from low (496 mg/d) to high (1437 mg/d) (see paragraph 2.75). These findings may not be applicable to children (who have higher calcium requirements because of increased metabolism) or in patients with metabolic bone disease.

UK general population aged 11y and above

9.44 An RNI of 10 µg/d (400 IU) of vitamin D is proposed for the UK general population aged 11y and above. This is the average vitamin D intake (from natural food sources, fortified food or supplements) needed to achieve a serum 25(OH)D concentration ≥ 25 nmol/L during winter in 97.5% of the population. It refers to the average vitamin D intake over a period of time (e.g., 1 week) and takes account of day to day variations in intake.

9.45 The RNI assumes minimal sunshine exposure because the studies used to derive this figure were carried out in winter.

9.46 Although most people would be expected to synthesise vitamin D and naturally achieve a serum 25(OH)D concentration ≥ 25 nmol/L during the summer due to sun exposure, the NDNS and other studies indicate that a substantial proportion (7-53%) 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.

- *Pregnancy and lactation*

9.47 Data are not available to suggest the requirement for an additional increment during pregnancy and lactation. Therefore, the RNI proposed for the general UK population (10 µg or 400 IU/d) is also applicable to pregnant and lactating women.

UK general population aged under 11y

- *Infants and children under 4y*

9.48 Current RNIs for infants and children are 8.5 µg/d (340 IU) for ages 0-6m and 7 µg/d (280 IU) for ages 7m-3y (DH, 1991). The RNI for 0-6m is based on the amount of vitamin D in infant formula and the RNI for 7m-3y is based on recommendations from a previous COMA report (DHSS, 1988) that infants and young children (up to 2y) should receive supplementary vitamin D, then providing 7 µg/d (280 IU). It is uncertain how these figures were derived and the paucity of data does not support different vitamin D requirements for ages 0-6m and 7m-3y.

9.49 Since data are not available to relate serum 25(OH)D concentration in the infant clearly to current or long term health, *Safe Intakes*[101] rather than RNIs are proposed for ages 0 up to 4y. 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 *'judged to be a level or range of intake at which there is no risk of deficiency, and below a level where there is a risk of undesirable effects'* (DH, 1991).

9.50 Evidence from the *DNSIYC* shows that out of those infants (aged 5-11m) with a serum 25(OH)D concentration < 25 nmol/L (6%), none were receiving infant formula. Therefore, in pragmatic terms, setting a Safe Intake range of 8.5-10 µg/d (340-400 IU) for infants aged 0-11m accommodates current practice, determined by concentrations of vitamin D in infant formula. The proposed Safe Intake range is not additional to, but includes, vitamin D intakes obtained from infant formula.

9.51 There is currently no vitamin D RNI for exclusively breast fed infants because it was previously assumed that maternal vitamin D supplementation during pregnancy, together with intake from breast milk, would provide the infant with adequate vitamin D and sustain plasma 25(OH)D concentration during the period of exclusive breast feeding. Although evidence on exclusively breast fed infants is limited, the available data indicate that it is unlikely that an exclusively breast fed infant in the UK would maintain a serum 25(OH)D concentration ≥ 25 nmol/L for 6 months. Therefore the Safe Intake range recommended for non-breastfed infants is also recommended for exclusively breastfed infants and mixed-fed infants (those part breast-fed and part formula fed), from birth; i.e., the Safe Intake range of 8.5-10 µg/d (340-400 IU) is recommended for *all* infants aged 0-11 months.

9.52 A Safe Intake of 10 µg/d (400 IU), based on the RNI for the rest of the UK population, is recommended for children aged 1 up to 4y.

- *Children aged 4 up to 11y*

9.53 There is no evidence to suggest that the RNI for infants and children aged 4 up to 11y should be a different value to the RNI proposed for the UK population (10 µg/400 IU per day) aged 11y and above. An RNI of 10 µg/d (400 IU) is therefore also considered appropriate for this age group.

- *'At risk' groups*

9.54 Individuals in population groups '*at risk*' of having a serum 25(OH)D concentration < 25 nmol/L are those from ethnic groups with dark skin, those who are seldom outdoors and those wearing concealing clothing. A subset of adults ≥ 65y may also be at risk of having a serum 25(OH)D

[101] 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).

concentration < 25 nmol/L due to spending less time outdoors, either because of frailty or being institutionalised.

9.55 An additional increment is not considered necessary for population groups who spend less time outdoors or wear concealing clothing because the recommendation for the proposed RNI to be applicable throughout the year is intended 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.

9.56 Lower serum 25(OH)D concentrations have been observed in ethnic groups with dark skin. However, dark 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., 2014) (see paragraphs 5.17-5.19). It is also not known if the lower serum 25(OH)D concentrations observed in people with dark skin are associated with any adverse health outcomes since the evidence on vitamin D and 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 dark skin is higher than the RNI of 10 µg/d (400 IU) 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 (400 IU) is considered appropriate to cover the needs of ethnic groups within the UK population.

9.57 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 proposed for the general population.

9.58 If the proposed RNI is achieved by almost all of the UK population, the current distributions of vitamin D intakes and serum 25(OH)D concentrations would shift to the right with an increase in mean/median intakes and serum 25(OH)D concentrations. It is unlikely, however, that this would lead to those with intakes at the top end of the distribution reaching vitamin D intakes/serum 25(OH)D concentrations that might pose a risk of adverse effects. Findings from a study (Allen et al., 2015) which used NDNS (2008-2010) data (n=2127) to model the effect of vitamin D intakes resulting from different fortification scenarios on population groups at risk of vitamin D deficiency[102], 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 UL[103] (EFSA, 2012).

[102] 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.

[103] 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 (1000 IU/d); 11-17y & adults, 50 µg/d (2000 IU/d). The ULs were revised by EFSA in 2012: < 1y, 25 µg/d (1000 IU/d); 1-10y, 50µg/d (2000 IU/d); 11-17y & adults, 100 µg/d (4000 IU/d). The revised ULs were considered appropriate by the COT and accepted as the ULs for the UK (COT, 2014).

Summary & conclusions

9.59 The RNI for vitamin D recommended for the UK population is based on protection of musculoskeletal health. The RNI represents the amount of a nutrient that is enough, or more than enough, to meet the needs of 97.5% of the population.

9.60 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.

9.61 Sunlight UVB exposure could not be taken into account in setting the RNI because it was not possible to quantify the contribution it made to serum 25(OH)D concentrations within the general population.

9.62 The process of translating the target serum 25(OH)D concentration of ≥ 25 nmol/L into an RNI for vitamin D was based on dose response RCTs carried out during winter when UVB radiation is absent or minimal.

9.63 An RNI of 10 µg/d (400 IU/d) of vitamin D is recommended for the UK population aged 4y and above. This represents the average amount of vitamin D (from natural food sources, fortified foods or supplements) that is needed to achieve a serum 25(OH)D concentration ≥ 25 nmol/L during winter in 97.5% of the population. It refers to the average vitamin D intake over a period of time and takes account of day to day variations in intake.

9.64 An RNI of 10 µg/d (400 IU/d) vitamin D for the general UK population aged 4y and over is a change to current advice.[104]

9.65 The RNI assumes minimal sunshine exposure.

9.66 As a precaution, it is recommended that the RNI is applicable throughout the year to protect population groups and unidentified individuals in the UK with a serum 25(OH)D concentration < 25 nmol/L in the summer.

9.67 The RNI of 10 µg/d (400 IU/d) recommended for the general UK population (4y and above) includes pregnant and breast feeding women; i.e., a separate recommendation is now not required for pregnant and lactating women. This is a change from previous advice.

9.68 The RNI of 10 µg/d (400 IU/d) makes provision for and therefore includes *at risk* population groups (frail older adults and other individuals not spending substantial time outdoors, those wearing concealing clothing; and people from ethnic groups with dark skin).

9.69 A Safe Intake range of 8.5-10 µg/d (340-400 IU/d) is proposed for infants aged 0-11m and includes those who are exclusively breast-fed and those who are breast fed and part-formula fed, from birth. The Safe Intake recommended for exclusively breastfed infants is a change to previous advice.

9.70 A Safe Intake of 10 µg/d (400 IU/d) is proposed for children aged 1 to < 4 y.

[104] Current advice is that no dietary intake is necessary for individuals aged 4-64y with adequate exposure to sunlight.

TABLE 3: Estimated dietary requirements for vitamin D at selected percentiles to maintain serum 25(OH)D above selected concentrations during winter

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

Serum 25(OH)D (nmol/L)	50th percentile[3]		90th percentile		95th percentile		97.5th percentile	
	µg/d (IU/d)							
Men and women aged 20-40y (n=215)[1]								
> 25	-		2.7	(108)	5.9	(236)	**8.7**	**(348)**
> 30	-		7.6	(304)	10.9	(436)	13.7	(548)
> 40	4.1	(164)	15.5	(620)	18.8	(752)	21.7	(868)
> 50	10.2	(408)	21.7	(868)	25.0	(1000)	28.0	(1120)
Free-living men and women aged ≥ 64y (n=225)[2]								
> 25	-		2.6	(104)	5.8	(232)	**8.6**	**(344)**
> 30	-		6.2	(248)	9.4	(376)	12.2	(488)
> 40	1.3	(52)	12.7	(508)	16.0	(640)	18.8	(752)
> 50	7.1	(284)	18.4	(736)	21.8	(872)	24.7	(988)
Adolescent girls aged 11y (n=144)[1]								
> 25	0.2	(8)	5.5	(220)	7.0	(280)	**8.3**	**(332)**
> 30	2.2	(88)	7.8	(312)	9.0	(360)	10.3	(412)
> 40	6.3	(252)	11.7	(468)	13.2	(528)	14.5	(580)
> 50	10.4	(416)	15.8	(632)	17.3	(692)	18.6	(744)

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

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

[3] The vitamin D intake that will maintain serum 25(OH)D concentrations above the indicated cut-off level during winter in 50% of adults aged 20-40y, adults aged ≥ 64y and in girls aged 11y.

10. Overall summary and conclusions

Background

10.1 The two major forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The principal sources of vitamin D are sunlight exposure (skin synthesis) and foods or dietary supplements (containing either vitamin D_2 or D_3). Between the months of April and September, skin synthesis is the main source of vitamin D for most people. Vitamin D_3 is the only form produced cutaneously. Vitamin D_2 is formed in fungi and yeast by UVB exposure of ergosterol.

10.2 Dietary sources of vitamin D include 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 D_3 (e.g., oily fish, red meat, egg yolk). Animal products (e.g., meat, fat, liver, kidney) also contain the vitamin D metabolite, 25(OH)D. Wild mushrooms are a rich source of vitamin D_2. Fortified foods (e.g., breakfast cereals, fat spreads) and dietary supplements contain either vitamin D_2 or D_3. Dietary sources are essential when the amount of sunlight containing UVB light is limited (e.g., in winter) or exposure to sunlight containing UVB light is restricted (e.g., lack of time spent outdoors or little skin exposure).

10.3 UK DRVs for vitamin D, set by 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 RNI[105] was not set for individuals aged 4-64y *'living a normal lifestyle'.* RNIs were set only for certain population subgroups considered to be at risk of vitamin D deficiency: infants 0-6 months (8.5 µg/340 IU per day); infants and children 7m–3y (7 µg/280 IU per day); pregnant and breast-feeding women (10 µg/400 IU per day), adults aged 65y and above (10 µg/400 IU per day), those with limited exposure to sunlight (e.g., confined indoors or wearing concealing clothing) and people of Asian ethnic origin (10 µg/400 IU per day).

10.4 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 previously considered by SACN in 2007. It was concluded that there was insufficient evidence, at that time, to amend existing advice and that evidence on the relationship between vitamin D and non-musculoskeletal health was inconclusive.

10.5 In 2010, SACN decided to review the DRVs for vitamin D because a substantial amount of data had accumulated since 2007, including a comprehensive report by the IOM in the USA[106] (IOM, 2011), which provided an important resource for consideration of the evidence.

Terms of reference

10.6 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 terms of reference of the current SACN review on vitamin D was: *to review the Dietary Reference Values for vitamin D and make recommendations.* The key issues considered were:

[105] The RNI represents the amount of a nutrient that is likely to meet the needs of 97.5% of the population.
[106] *Dietary Reference Intakes for Calcium and Vitamin D*.

a) biochemical indicators of vitamin D status and the validity of the values used to assess risk of deficiency and excess;

b) association between vitamin D status and health outcomes at all life stages and the effects of biological modifiers;

c) 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;

d) potential adverse effects of high vitamin D intakes; and

e) 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.

10.7 Data from RCTs, then prospective studies, when available, were preferred for setting DRVs but data from other study types were also considered (including case-control, cross-sectional studies and case reports).

Biology & metabolism

10.8 The first step in endogenous vitamin D synthesis is the conversion by solar UVB radiation of 7-DHC to previtamin D in the skin. The dose response is not linear, which means that longer exposures do not lead to proportionally greater previtamin D synthesis. Previtamin D_3 is thermodynamically unstable and is converted at body temperature to vitamin D_3 which enters the circulation and is transported to the liver bound to vitamin D binding protein.

10.9 Dietary vitamin D is lipid soluble and is incorporated within enterocytes into chylomicrons that are secreted into lymph and transported through the lymphatic system to the systemic circulation to the liver.

10.10 Vitamin D is converted to its active metabolite $1,25(OH)_2D$ in two hydroxylation steps: firstly to 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.

10.11 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.

10.12 Polymorphisms in genes encoding proteins involved in vitamin D metabolism have been identified which might influence serum 25(OH)D concentrations and functional outcomes but the nutritional implications of such findings is not clear.

Biomarkers of exposure

10.13 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 skin synthesis (including season, latitude, clothing, skin type).

10.14 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.

10.15 Quantification of serum 25(OH)D concentration is influenced by the analytic methodology. 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 accuracy, specificity, sensitivity and better reproducibility.

10.16 The main limitations associated with the antibody-based 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

10.17 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.

10.18 The amount of vitamin D synthesised in the skin depends on skin exposure to solar 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.

10.19 In the UK, sunlight is not effective for vitamin D synthesis when the sun is low in the sky (sunrise or sunset). The most effective time for vitamin D synthesis is at midday.

10.20 There is a well observed seasonal cycle in serum 25(OH)D concentrations in the UK. Solar radiation is greater in the summer because the sun is much higher, the days are longer and the weather less cloudy. Solar radiation is reduced during winter because of low solar elevation, short days and cloudy skies. The small amount of UVB radiation in winter sunlight is insufficient to initiate synthesis of any biologically relevant amounts of vitamin D. Sunlight-induced vitamin D synthesis in white-skinned populations becomes effective from late March/early April with maximum concentrations observed in September after a summer of exposure. Serum 25(OH)D concentration decreases from October onwards throughout the winter months.

10.21 UVB (as a proportion of UVR) lessens with increasingly northern latitudes. 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.

10.22 The amount of 7-DHC 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.

10.23 The pigment melanin absorbs some of the UVB radiation which would otherwise be absorbed by 7-DHC. 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 dark 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

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

Musculoskeletal health outcomes

10.25 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.

10.26 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 wide variation in the serum 25(OH)D concentration at which rickets was present and a clear threshold serum 25(OH)D concentration above which there is no risk could not be identified; however, in the majority of studies considered, individual or mean serum 25(OH)D concentration was < 25 nmol/L in children with rickets. Although the risk of rickets is increased at serum 25(OH)D concentration < 25 nmol/L, this concentration is not a clinical threshold diagnostic of the disease and most children in the general population with a serum 25(OH)D concentration < 25 nmol/L will not develop rickets. The concentration below which there is increased risk of rickets specifically due to vitamin D is uncertain. The presence of rickets at serum 25(OH)D concentrations ≥ 25 nmol/L might be caused by calcium deficiency.

10.27 Evidence on vitamin D and osteomalacia is limited and drawn mainly from case reports. There is no clear serum 25(OH)D threshold concentration below which risk of osteomalacia is increased but was associated with serum 25(OH)D concentration < 20 nmol/L in all the studies considered.

10.28 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 serum 25(OH)D concentration during pregnancy and bone health indices in the fetus/newborn but the physiological significance of this is not known. Beneficial effects of vitamin D supplementation were also observed on bone health indices at some skeletal sites in adults aged ≥ 50y. Evidence on vitamin D and bone health indices in infants, children and adolescents and adults < 50y was either inconsistent or insufficient to draw conclusions.

10.29 Cohort studies suggest an association between increased serum 25(OH)D concentration and decreased fracture risk in adults ≥ 50y; evidence from RCTs, however, suggests vitamin D plus calcium supplementation is more effective than vitamin D alone in reducing fracture risk. On balance, vitamin D supplements alone appear to have no effect on fracture risk in older men and women. Evidence on vitamin D supplementation or serum 25(OH)D concentration on stress fracture risk in younger age groups is insufficient to draw firm conclusions.

10.30 Evidence from RCTs suggest that vitamin D supplementation may improve muscle strength and function in adults < 50y with mean serum 25(OH)D concentration < 30 nmol/L. In adults ≥ 50y, the evidence is mixed but suggests, overall, beneficial effects on muscle strength and function with mean baseline serum 25(OH)D concentrations across a range of values.

10.31 Evidence on vitamin D and falls is mixed but, overall, suggests a beneficial effect of vitamin D supplementation in reducing fall risk in community dwelling adults ≥ 50y of age with baseline serum 25(OH)D concentrations across a range of values. Two RCTs reported an increase in fall risk with high dose vitamin D supplementation whether monthly or annually but high dose and delivery on a monthly or annual basis may produce different effects from daily lower dose supplementation.

Non-musculoskeletal health outcomes

10.32 Evidence on vitamin D and a range of non-musculoskeletal health outcomes was considered: reproductive health (on maternal & newborn outcomes), cancers, CVD, hypertension, all-cause mortality, immune modulation (asthma, atopic disorders, multiple sclerosis, type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis), infectious diseases (TB, acute respiratory tract infections), neuropsychological functioning (cognitive function, dementia, autism, depression, schizophrenia), age-related macular degeneration and oral health.

10.33 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. Findings from RCTs of vitamin D supplementation and non-musculoskeletal health outcomes are inconsistent. 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

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

10.35 There was wide variability in mean and individual serum 25(OH)D concentrations associated with increased risk of rickets, osteomalacia or falls or improvement in muscle strength and function. There were also a number of limitations in the data, including the use of predefined cut-offs based on different criteria for vitamin D deficiency. Interpretation of the data was also 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.

10.36 The evidence considered suggests, overall, that risk of poor musculoskeletal health increases at serum 25(OH)D concentrations below about 20-30 nmol/L. The number of uncertainties in the data makes it difficult to identify a specific serum 25(OH)D threshold concentration between this range, 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 concentration is not diagnostic of disease but indicates the serum 25(OH)D concentration below which the risk of poor musculoskeletal health is increased at a population level. It therefore represents a *population protective* concentration; i.e. the concentration that the majority (97.5%) of individuals in the population should achieve, or be above, in order to protect their musculoskeletal health.

Potential adverse effects of high exposures to vitamin D

10.37 Potential adverse effects of high vitamin D intakes were considered by the COT. 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 ULs[107] for vitamin D recommended by EFSA are 100 µg/d (4000 IU) for adults and children aged 11-17y, 50 µg/d (2000 IU) for children aged 1-10y and 25 µg/d (1000 IU) for infants. These ULs were considered appropriate by the COT. The UL may not apply to individuals with certain medical 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.

10.38 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 at 3 month or longer intervals would not be expected to cause any adverse effects but there is greater uncertainty at higher doses. For infants, limited data suggest that toxicity could occur at single vitamin D doses ≥ 15,000 µg (600,000 IU).

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

Vitamin D intakes

10.39 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) (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 were drawn from the Low income Diet and Nutrition Survey (LIDNS). Data on pregnant women were obtained from UK based cohort studies[108].

10.40 The NDNS shows that mean dietary intakes of vitamin D from all sources (including dietary supplements) were approximately 7.7-10 µg/d (308-400 IU/d) for non-breast fed infants aged 4-11m and 3.9 µg/d (156 IU/d) for 12-18m; 3.5-3.8 µg/d (140-152 IU/d) for breast fed infants aged 4-11m and 2.6 µg/d (104 IU/d) for those aged 12-18m; 2.3-2.7 µg/d (92-108 IU/d) for ages 1.5-18y; and 3.6-5.1 µg/d (144-204 IU/d) for 19-65+y. For institutionalised adults, mean daily vitamin D intake from all sources was 3.9 µg (156 IU/d) (39% of RNI) for men and 3.4 µg (136 IU/d) (34% of RNI) for women.

10.41 Infant formula was the main contributor to vitamin D intake for infants aged 4-18m. For all age groups aged > 18m (except children aged 1.5-3y), meat and meat products were the main sources of dietary vitamin D providing 23-35% of intake. For children aged 1.5-3y the major contributor was milk (and milk products), providing 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.

[107] UL - 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.

[108] Nationally representative data are not available for pregnant women.

Serum/plasma 25(OH)D concentration

10.42 In the DNSIYC and NDNS annualised[109] mean plasma 25(OH)D concentrations in the UK were: 64-69 nmol/L for ages 5-18m; 58 nmol/L for ages 1.5-3y[110]; 50 nmol/L for ages 4-10y; 43-45 nmol/L for ages 11-65+y. The percentage with plasma 25(OH)D concentrations < 25 nmol/L was: 5-11m, 6%; 12-18m, 2%; 1.5-3y, 7.5%; 4-10y, 12-16%; 11-18y, 20-24%; 19-64y, 22-24%; 65+y, 17-24%. 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.

10.43 For low income groups, annualised mean plasma 25(OH)D concentrations were 40-44 nmol/L in children aged 11-18y[111] and 43-53 nmol/L in adults aged ≥ 19y. The percentage with plasma 25(OH)D concentration < 25 nmol/L was: 8% of boys and 23% of girls aged 11-18y and 14-25% of adults aged ≥19y.

10.44 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 winter was: children (4-10y), 31%; older children and adolescents (11-18y), 40%; adults aged 19-64y, 39%; and adults aged ≥ 65y, 29%. The proportion in the summer was: 2% of children (4-10y); 13% of older children and adolescents (11-18y); 8% of adults aged 19-64y; and 4% of adults aged ≥ 65y.

10.45 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 ≥ 25 nmol/L in *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. Data from UK based cohort studies in pregnant women have reported mean/median serum 25(OH)D concentrations of 34-53 nmol/L in summer and 26-34 nmol/L in winter. The percentage with concentrations < 25 nmol/L ranged from 25-29% in the summer to 49-76% in the winter.

10.46 An HSE analysis by ethnicity showed that annualised mean serum 25(OH)D concentration was higher in white adults aged ≥ 16y (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 2y) reported that the mean serum 25(OH)D concentration (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 lower mean serum 25(OH)D concentrations (in every season) in South Asian women compared to white women and a higher percentage with serum 25(OH)D concentration < 25 nmol/L throughout the year (53% in summer, 75% in winter) compared with white women (0.4% in summer, 9.7% in winter).

[109] Average of reports from different months of the year.
[110] Based on small sample size (n=42).
[111] Sample size in boys aged 8-10 years was too small (n=8) to be representative.

Review of DRVs

10.47 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.

10.48 Previously in the UK, an RNI (but not an EAR or LRNI) for vitamin D was set only for population groups at high risk of deficiency (DH, 1991). It was assumed that, for most people, the amount of vitamin D produced by exposure to sunlight containing UVB in the summer months would be adequate for achieving serum 25(OH)D concentrations ≥ 25 nmol/L during winter. It is now known that this is not the case.

10.49 In the current review, musculoskeletal health was used as the basis for setting DRVs for vitamin D. The available data were not sufficient to establish a distribution of serum 25(OH)D concentrations that would be required to set the 3 DRV estimates for vitamin D intake (i.e., LRNI, EAR, RNI) or to identify a clear threshold serum 25(OH)D concentration to support musculoskeletal health outcomes. However, the evidence overall suggests that risk of poor musculoskeletal health increases at serum 25(OH)D concentrations below 25 nmol/L. A serum 25(OH)D concentration ≥ 25 nmol/L was therefore considered to be a *'population protective level'*; i.e., the concentration that 97.5% of individuals in the UK should achieve, or be above, in terms of protecting musculoskeletal health.

10.50 A serum 25(OH)D concentration of ≥ 25 nmol/L was selected as the basis for deriving the RNI for vitamin D; i.e., the mean vitamin D intake required to achieve a serum 25(OH)D concentration ≥ 25 nmol/L by 97.5% of the population. The next step was to estimate the average dietary intake value that would achieve a serum 25(OH)D concentration ≥ 25 nmol/L 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 establishment of an EAR or LRNI. The average vitamin D intake refers to the mean or average intake over a period of time (e.g., 1 week) and takes account of day to day variations in vitamin D intake.

10.51 Sun exposure is the major source of vitamin D during the summer months for the majority of people in the UK and most achieve a serum 25(OH)D concentration > 25 nmol/L in summer. A modelling exercise was carried out to estimate the summer sunshine exposure required to maintain serum 25(OH)D concentrations ≥ 25 nmol/L during winter. However, it was not possible to quantify the sunlight exposure required in the summer months to maintain a winter serum 25(OH)D concentration of ≥ 25 nmol/L because of the number of factors that affect endogenous vitamin D synthesis, storage and utilisation. It was, therefore, not possible to take account of sunlight exposure in setting the RNI.

10.52 The RNI for vitamin D was estimated by modelling data from individual vitamin D RCTs in adults (men & women, 20-40 y and ≥ 64y) and adolescent girls (11 y). The RCTs were conducted in winter so that skin production of vitamin D was minimal. The modelling exercise indicated that the estimated average daily vitamin D intake required to maintain serum 25(OH)D concentration ≥ 25 nmol/L in winter by 97.5% of the population is 12 µg (480 IU) based on serum 25(OH)D analysis by LC-tandem MS or 9 µg (360 IU) 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 µg/d (400 IU).

10.53 An RNI of 10 µg/d (400 IU/d) is recommended for the general UK population aged 11y and above. The RNI assumes minimal sunshine exposure because the RCTs used to derive this figure were conducted in winter. This is the average amount needed to achieve a serum 25(OH)D concentration ≥ 25 nmol/L during winter in 97.5% of the population.

10.54 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 8.45-8.47). Since it is not possible to identify these individuals, it is recommended that the RNI is applicable to the UK population 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 is to ensure coverage of 97.5% of the population throughout the year.

10.55 The RNI of 10 µg/d (400 IU) recommended for the general UK population includes pregnant and lactating women. The previous COMA recommendation (DH, 1991) that pregnant and lactating women should receive supplementary vitamin D to achieve an intake of 10 µg/d (400 IU) has been superseded by the RNI of 10 µg/d (400 IU/d) now recommended for the general UK population (aged 4y and above); i.e., a separate recommendation is now not required for pregnant and lactating women.

10.56 Data were not available to allow direct determination of the vitamin D intake required to reach serum 25(OH)D concentrations ≥ 25 nmol/L in infants and children under 11y. 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 and the RNI of 10 µg/d (400 IU) recommended for the UK population was considered appropriate for children aged 4 up to 11y.

10.57 Since data are not available to clearly relate serum 25(OH)D concentration in the infant to current or long term health, S*afe Intakes*[112] rather than RNIs are recommended for infants and children aged up to 4y. Safe Intakes are based on a precautionary approach and reflect the insecurities of the data.

10.58 A Safe Intake range of 8.5-10 µg/d (340-400 IU), based on concentrations of vitamin D in infant formula, is recommended for infants aged 0-11m.

10.59 There is currently no vitamin D RNI for exclusively breastfed infants because it was previously assumed that maternal vitamin D supplementation during pregnancy and then 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 unlikely that an exclusively breastfed infant in the UK would maintain a serum 25(OH)D concentration ≥ 25 nmol/L for 6 months. Therefore the Safe Intake range of 8.5-10 µg/d (340-400 IU) is recommended for all infants, including those who are exclusively breast fed and those who are breastfed and part-formula-fed, from birth.

10.60 A Safe Intake of 10 µg/d (400 IU), based on the RNI for the UK population, is recommended for infants and children aged 1-3y.

10.61 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

[112] 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).

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 to the RNI was not considered necessary for these *'at risk'* population groups because the recommendation for the RNI to be applicable all year is to take account of individuals with minimal sunshine exposure, including those most at risk.

10.62 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.

10.63 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

11.1 Serum 25(OH)D concentration is an indicator of exposure to vitamin D (from skin synthesis and dietary intake). In order to protect musculoskeletal health, it is recommended that the serum 25(OH)D concentration of all individuals in the UK should not fall below 25 nmol/L at any time of the year.

11.2 In the UK, individuals in 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 time outdoors (e.g., frail and institutionalised people) or habitually wearing clothing that covers most of the skin while outdoors and those from minority ethnic groups with dark skin.

11.3 It is not possible to make any recommendations regarding the amount of sunlight exposure that would be required during the summer to maintain serum 25(OH)D concentration ≥ 25 nmol/L in 97.5% of the UK population during the following winter because of the number and complexity of factors that affect endogenous vitamin D production.

11.4 An RNI for vitamin D, of 10 µg/d (400 IU/d), is recommended for the UK population aged 4y and above. This is the average amount needed by 97.5% of the population to maintain a serum 25(OH)D concentration ≥ 25 nmol/L when UVB sunshine exposure is minimal. It refers to average intake over a period of time (e.g., a week) and takes account of day to day variations in vitamin D intake.

11.5 The RNI of 10 µg/d (400 IU/d) proposed for the general UK population (aged 4y and above) includes pregnant and lactating women and population groups at increased risk of having a serum 25(OH)D concentration < 25 nmol/L. A separate RNI is not required for these groups. This is a change from previous advice.

11.6 Data are insufficient to set RNIs for infants and children aged under 4y. As a precaution, a 'Safe Intake' of vitamin D is recommended for these ages: in the range 8.5-10 µg/d (340-400 IU/d) for ages 0 up to 1y (including exclusively breast fed and partially breast fed infants, from birth); and 10 µg/d (400 IU/d) for ages 1 up to 4y. The recommendation for exclusively breast fed infants is a change from previous advice.

11.7 It is recommended that the RNI/Safe Intakes are applicable throughout the year, as a precautionary measure, to cover population groups in the UK identified to be at risk of having a serum 25(OH)D concentration < 25 nmol/L (see paragraph 11.2 above) as well as unidentified individuals in the population at risk of having a serum 25(OH)D concentration < 25 nmol/L in summer.

11.8 The RNI/Safe Intake for vitamin D refers to intakes from *all* dietary sources: natural food sources; fortified foods (including infant formula milk); and supplements. Since it is difficult to achieve the RNI/Safe Intake from natural food sources alone, it is recommended that the Government gives consideration to strategies for the UK population to achieve the RNI of 10 µg/d (400 IU/d) for those aged 4y and above and for infants and younger children to achieve a Safe Intake in the range 8.5-10 µg/d (340-400 IU/d) at ages 0 to < 1y and 10 µg/d (400 IU/d) at ages 1 to < 4y.

12. Research recommendations

12.1 RCT data are lacking on possible relationships between vitamin D and non-musculo-skeletal outcomes. Although a number of ongoing trials and five large RCTs are due to be completed over the next 3-4 years, a potential inherent limitation is that participants are not exclusively those with low serum 25(OH)D concentrations (i.e., < 20-30 nmol/L) at baseline, so any potential beneficial effects might be reduced. Future RCTs should therefore stratify participants, *a priori,* to include individuals with serum 25(OH)D concentrations < 20-30 nmol/L rather than relying on secondary subgroup analysis.

12.2 Gaps remain in the evidence on vitamin D and musculoskeletal health, especially in relation to calcium intakes. Future RCTs should separate effects of vitamin D alone and in combination with calcium on the musculoskeletal system throughout the life course.

12.3 RCTs are also needed to clarify whether vitamin D doses above 10 µg/d (400 IU/d) would offer benefits additional to musculoskeletal health.

12.4 More sensitive biomarkers of vitamin D status and function suitable for use in population health studies and trials should be developed.

12.5 Further research is also required in the following areas:

i. the influence of body weight/composition on the response of serum 25(OH)D concentration to vitamin D exposure;

ii. whether adipose tissue or other tissues of the body act as a store or non-reversible sink for vitamin D and its metabolites;

iii. the effect of ageing on cutaneous vitamin D synthesis;

iv. the effects of serum 25(OH)D concentrations at the higher end of the distribution for the general UK population, especially older persons;

v. whether the interpretation of serum 25(OH)D concentration is affected by inflammation and the acute phase response;

vi. whether there are differences in dietary vitamin D requirements of ethnic groups in the UK.

12.6 Future national surveys should focus on measuring serum 25(OH)D concentrations in population groups for which there is a lack of national data (infants and children under 4y of age, exclusively breast fed infants from birth, pregnant and lactating women and minority ethnic groups). Future prospective studies should examine the determinants of maternal and early-life serum 25(OH)D concentration.

12.7 Food-based strategies for the UK general population (particularly pregnant women, children and ethnic subgroups) to achieve the RNI/Safe Intake for vitamin D should be explored and developed.

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Annexes

ANNEX (1)

SACN working procedures

Vitamin D Working Group

1. The Vitamin D Working Group (WG) was established in 2011 and met 15 times: twice in 2011, four times in 2012, four times in 2013, three times in 2014 and two times in 2015. The final and 15th meeting of the WG was to consider the responses received to the scientific consultation on the draft report (see below). The minutes of all the meeting are available on the GOV.UK website at the following link: https://www.gov.uk/government/groups/scientific-advisory-committee-on-nutrition#vitamin-d-working-group

SACN

2. The draft report was considered by the full committee in November 2014, June 2015 and February 2016.

Consultation

3. The draft report was posted on the SACN website from 22 July to 23 September 2015 and interested parties were invited to submit comments relating to the scientific aspects of the report.

4. Responses were received from the following organisations and individuals.

    1. Alliance for Natural Health International
    2. Association for Nutrition
    3. Aziz, M
    4. Birmingham Vitamin D Steering Group
    5. Bone Research Society
    6. British Dietetic Association
    7. British Nutrition Foundation
    8. British Retail Consortium
    9. Children's Food Trust
    10. Collins, Dr S (CChem MRSC)
    11. Council for Responsible Nutrition UK
    12. Fischer, M (Systems Biology Laboratory, Vitamin D Association)
    13. Food & Drink Federation
    14. Greenbaum R (A & B)

15. Harvey N (Professor of Rheumatology & Clinical Epidemiology) & C Cooper (Professor of Rheumatology & Director) (Southampton University)
16. Health Food Manufacturers' Association
17. Hewison, M (Professor of Molecular Endocrinology, Birmingham University)
18. Hogler, Dr W (Global consensus recommendations for prevention & management of rickets)
19. Huish, S (Specialist Dietitian, Coventry & Warwickshire NHS Trust)
20. Internis Pharmaceuticals Ltd.
21. Jacobs, Dr B (Consultant Paediatrician, Royal National Orthopaedic Hospital)
22. Macdonald, Professor H (University of Aberdeen)
23. Marshall, A (Paediatric trainee)
24. Martineau, A (Professor of Respiratory Infection & Immunity, Barts & The London School of Medicine & Dentistry)
25. Mawjes, Ms Sile
26. MRC Human Nutrition Research
27. National Osteoporosis Society
28. Newton-Bishop, J (Professor of Dermatology, Leeds Institute of Cancer & Pathology)
29. NHS Health Scotland
30. Northern Ireland Centre for Food & Health (NICHE), Ulster University
31. Norval, M (Professor Emeritus, University of Edinburgh Medical School)
32. Oliver, T (Professor Emeritus in Oncology, Bart's Cancer Institute)
33. Proprietary Association of Great Britain
34. Rayner, J (Paediatric Dietitian, Bedford Hospital NHS Trust)
35. Rhein, H (General Practitioner, Edinburgh)
36. Rhodes, L (Professor of Experimental Dermatology, University of Manchester)
37. Royal College of Physicians
38. Sexton, S (Specialist Medicines Management Dietitian, Bristol CCG)
39. Tagliaferri S, De Guiseppe R, Cena H; University of Palma & University of Pavia
40. Taylor, H (Clinical Effectiveness Pharmacist, NHS Sheffield CCG)
41. Thompson, P
42. Waitrose
43. Wales Dietetic Leadership Advisory Group (WDLAG) & Public Health Dietitians in Wales (PHDiW)
44. Watson, I

5. The responses can be viewed in full on the GOV.UK website at the following link: https://www.gov.uk/government/groups/scientific-advisory-committee-on-nutrition

ANNEX (2)

Studies considered in relation to vitamin D and health outcomes

Musculoskeletal health outcomes

Adults 50 years and above

Non-musculoskeletal health outcomes

Pregnancy & lactation

Cancers

Rickets

Table 1: Case reports of rickets

Study/year/country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Ca intake/other comments
Moncrieff & Fadahunsi (1974) Derby, UK	Infant (female) - born to mother (S Asian) who presented with biochemical & clinical evidence of osteomalacia (see table 6)	Clinical, biochemical (low serum calcium and raised alkaline phosphatase) and radiological features of rickets	25(OH)D: 20.7 Ca: 67 (at 4 days)	Ca intake not reported
Iqbal *et al* (1994) Leicester, UK	Case 1- Male, age 2 m, presented with hypocalcaemic fits Case 2 - Female, age 12 m, presented with failure to thrive	Wrist x-rays.	Case 1: 25(OH)D: <2.5/Ca: 43 Case 2: 25(OH)D: 5/Ca: 85	Ca intake not reported.
Train *et al* (1995) London, UK	2 black, exclusively breast-fed infant males. Case 1 - hypocalcaemic stridor (age 5 m) Case 2 - respiratory distress (age 7 m)	X-ray examinations	Case 1 25(OH)D: < 2.5/Ca: 46 Case 2: Vitamin D3: 6/Ca: 36	Ca intake not reported.
Mughal *et al* (1999) UK	Exclusively breastfed infants born in UK (ethnic origin: Palestine, Gambia, Libya, Saudi Arabia, Iran, Algeria) Case 1 – Male, age 21 m Case 2 – Female, age 10 m Case 3 – Female, age 20 m Case 4 – Female, age 15 m Case 5 – Female, age 28 m Case 6 – Female, age 16 m	Clinical signs & symptoms: bow legs, rickety rosary, swelling of ends of long bones, frontal bossing of skull, delayed dentition, poor growth & slow motor development. Radiological features: generalised osteopenia, widening of growth plates, cupping of metaphyseal regions of long bone.	Case 1 – 25(OH)D: 10.7/Ca: 68 Case 2 – 25(OH)D: 18.7/Ca: 87 Case 3 – 25(OH)D: 3.5/Ca: 56 Case 4 – 25(OH)D: 11/Ca: 87 Case 5 – 25(OH)D: 14/Ca: 87 Case 6 – 25(OH)D: 11.5/Ca: 87	Ca intake not reported.
Ashraf & Mughal (2002) Manchester, UK	All of Pakistani ethnic origin Case 1 – age 15 m Case 2 – age 8 m Case 3 – age 9 m	Examined for deformities & swelling of the metaphyses due to rickets. Children with clinical signs of rickets had wrist x-ray.	Case 1 – 25(OH)D: 25/Ca: 92 Case 2 – 25(OH)D: 40/Ca:96 Case 3 – 25(OH)D: 32.4/ Ca: 100	Ca intake not reported.

Study/year/country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Ca intake/other comments
Crocombe *et al* (2004) Manchester, UK	9 cases aged 11-17 y (ethnic origin: Iran, India, Afghanistan, Pakistan)	Presented with symptoms of vitamin D deficiency, including lower limb pains, difficulty in walking or climbing stairs, carpopedal spasms & hypocalcaemic convulsions. Clinical signs included inability to stand up from squatting position due to proximal myopathy, bowed legs & knock-knees. 3 cases (1, 6, 9) with radiological changes incl. widening & fraying of metaphyses.	25(OH)D / Ca Case 1 <5 / 56 Case 2 <5 / 76 Case 3 25 / 79 Case 4 9 / 90 Case 5 <5 / 80 Case 6 7.5 / 80 Case 7 5.5 / 59 Case 8 <5 / 71 Case 9 <5 / 70	Ca intake not reported.
Odeka & Tan (2005) Oldham, UK	3 cases aged 15-19 m	Radiological evidence.	25(OH)D < 25 Ca concentration not reported	Ca intake not reported.
Kamien & Harris (2007) Perth, Australia	2 breast-fed female twins aged 10 m. Sudanese ethnicity Admitted to hospital with failure to thrive and massive splenomegaly.	X-rays of chest, hand & knee showed clinical rickets: rachitic rosary, large anterior fontanelle, no limb deformities, decreased muscle bulk and tone.	25(OH)D: 34/Ca: 88	Ca intake not reported.
Williams *et al* (2008) Boston, USA	African American child, age 11 months, diagnosed with rickets at well-child appointment.	Findings of florid rickets from wrist and knee radiographs: osteopenia, minimal fraying of distal ulnar and distal femoral regions and proximal fibular metaphyses.	25(OH)D: < 17.5/Ca: 97	Ca intake not reported.
Brown *et al* (2009) Washington, USA	Hospital database (1997-2007) searched for infants with vitamin D deficiency plus dilated cardiomyopathy (DCM). 4 infants (3 male, 1 female), age 4-10 m Exclusively breastfed; no vitamin D supplementation; African American descent.	Biochemical laboratory evidence consistent with hypocalcaemic rickets. Radiographic evidence consistent with rickets, including rachitic beads on ribs, fraying and cupping of metaphyses, and cardiomegaly.	25(OH)D: / Ca Case 1: 13 / 58 Case 2: 7 / 46 Case 3: 4 / 47 Case 4: <13 / 63	Ca intakes not reported.
Holick *et al* (2009) Mass. USA	Boy aged 9 m with generalised seizure and bulging fontanelle. Ethnic origin: East Africa.	Demineralisation present, rachitic rosary & other characteristic radiographic manifestations. Hypocalcaemia, hypophosphatemia, elevated ALP.	25(OH)D: 40	Ca intakes not reported.
Bhakhri & Debata (2010) New Delhi, India	Infant aged 10 m. Presented with marked pallor, abdominal distension, poor weight gain, delayed development.	Features of rickets observed: wide-open anterior fontanel, rosary, wrist widening, Harrison sulcus.	25(OH)D: 22.5/Ca: 72	Ca intakes not reported.

Study/year/country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Ca intake/other comments
Brouwers *et al* (2010) Netherlands	2 brothers. South Asian ethnic origin, living in Netherlands (2 years) Patient 1 - age 12 y; presented with waddling gait, muscular weakness and bone pain Patient 2 -age 14y; presented with waddling gait and pain when climbing stairs	Biochemical, clinical & radiographic evidence.	Case 1 25(OH)D: 8/Ca: 76 Case 2 25(OH)D: 4/Ca: not reported	Case 1: 100 mg/d Case 2: 330 mg/d
Akin *et al* (2010) Kayseri, Turkey	Male aged 23 m. Presented with recurrent febrile convulsions.	Diagnosed as antiepileptic drug-induced Vitamin D deficiency rickets. Mild enlargement of wrists bilaterally but rachitic rosary, craniotabes, caput quadratum, & leg deformities not present.	25(OH)D: 4.5/Ca: 64	Ca intakes not reported.
Pearson *et al* (2010) California, USA	Male aged 16 m. Presented with weight loss, elevated alkaline phosphatase and PTH. Hispanic ethnicity.	Clinical features of rickets - bow legs & mild frontal bossing. Radiographs consistent with early signs of rickets: widening & irregular metaphyses of distal femur & proximal tibia.	25(OH)D: 4.5/Ca: 89	Ca intakes not reported.
Brinsmead *et al* (2011) Brisbane, Australia	Girl aged 12 m Ethnicity, Indian; born Australia.	Radiological appearance of wrist and knees consistent with rickets; tender and widened wrists; hypocalcaemia.	25(OH)D: 11.2/Ca: 69	Ca intakes not reported.
Zurlo & Wagner (2012) Livingstone, US	Male, aged 8 m. African American. Presented at Emergency Dept. with fever.	X-rays showed flaring of ribs at costochondral junction (rachitic rosary), fraying & cupping of distal radial & ulnar metaphyses plus biochemical evidence.	25(OH)D: below threshold for detection. Ca: concentration not reported	Ca intakes not reported.

Table 2: Observational studies on rickets

Study/year/country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Calcium intakes/other comments
Dunnigan *et al* (1981) Glasgow, UK	South Asian children (n=189) aged 5-17 y in single GP practice. Ethnicity: South Asian Grouped according to vitamin D supplement use: Regular: at least 2x week, Intermittent: less frequently None	Radiological signs of rickets Regular: 3 (5%) Intermittent: 8 (15%) None: 11 (14%) No. diagnosed with rickets **(**serum Ca <90) Regular: 6 (11%) Intermittent: 16 (30%) None: 31 (39%)	25(OH)D Regular (n=54): 20.9 (0.76) Intermittent (n=49): 17.48 (0.71) None (n=75): 16.08 (0.57) Ca concentration not reported	Ca intakes not reported.
Ladhani *et al* (2004) London, UK	Retrospective review of case records of children (n=65; 0-13y) diagnosed with hypocalcaemia or vitamin D deficiency rickets (1996-2001). 39 Asian, 24 Afro-Carribean, 2 Eastern European.	12/29 with hypocalcaemia had radiological evidence of rickets 35/36 without hypocalcaemia had radiological evidence of rickets. Vitamin D deficiency defined as plasma 25(OH)D < 25 nmol/L.	25(OH)D median (range) With hypocalcaemia: 5.0 (2.1-14) Without hypocalcaemia: 6.7 (2.7-14) Ca With hypocalcaemia: 54.4 (33.6-83.2) Without hypocalcaemia: 84.4 (52.8-99.6)	Ca intake not reported. Hypocalcaemia peak in Mar-Jul; rickets presentation throughout year.
Lazol, Cakan & Kamat (2008) Michigan, USA	Retrospective review of case reports (n=58) over 10 y with nutritional rickets as primary or secondary diagnosis in Michigan hospital. Mean age: 18 months (range 2 to 132m); Ethnicity: 81% African American; 14% Arabic	Only included cases when radiological findings supported by laboratory data. Common manifestations of rickets: 74% wide swollen joints, 64% rachitic rosary, 58% bow legs, 36% bone pain, 36% frontal bossing, 31% motor delay, 30% seizures, 25% fractures.	25(OH)D: 79% < 50 33% < 12.5 Ca concentration not reported.	Ca intakes not reported.
Rajah *et al* (2008) Abu Dhabi, United Arab Emirates	Retrospective review of patients diagnosed with nutritional rickets (n=31) between June 2000-December 2003. 16 children with rickets diagnosis: 8 with 25OHD deficiency, 8 with Ca deficiency. Mean age Vit D deficiency group = 14.8 m ± 3.15 Ca deficiency group = 19.8 m ± 2.31	Any patient with combination of clinical and X-ray features in the presence of elevated alkaline phosphatase. Clinical features included widened wrists, frontal bossing, bowing of the legs and costochondral thickening.	<u>Vitamin D deficiency group</u>: 25(OH)D: 17.27 (4.65)/Ca: 91.6 (5.2) <u>Calcium deficiency group</u>: 25(OH)D: 44.30 (17.83)/Ca: 93.2 (7.6)	Ca intakes not reported

Study/year/country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Calcium intakes/other comments
Banajeh (2009) Sana'a, Yemen	Prospective cohort study (n=79); Age: 2-59 m	2 or more of following: rosary beads, craniotabes, frontal bossing, Harrison's sulcus with pigeon chest, wide anterior fontanel, widening of epiphysis, bowing of legs, delay dentition, double malulous. Considered rachitic if also had radiological signs of osteopaenia of upper arm bones & widening of costochondral junctions.	**25(OH)D:** 63% (50) > 30 37% (29) < 30 63% had clinical rickets. Of those with 25(OH)D < 30, 79% rachitic (radiological signs).	Ca intakes not reported.
Ekbote *et al* (2010) Pune, India	Children (n=111) living in the slums; mean age 2.6 y. Divided into following groups Group A: 50 '*outdoor toddlers'* not attending crèche Group B: 61 '*indoor toddlers'* attending crèche Sunlight exposure (no. of toddlers): Group A: 4<30min; 36>60min Group B: 24<30min;18>60min	Clinical signs, confirmed by radiographs: delayed closure of fontanel, frontal bossing, dental enamel hypoplasia, rickety rosary, swelling of wrists, knees and ankles, knock knees and bow legs. *Clinical signs of rickets:* Group A: none Group B: 10	Group A: Males: 95.9 (91.6) Females: 130.2 (67.7) Group B: Males: 14 (32) Females: 5.2 (21.1)	**Median:** Group A Males: 216 mg/d Females: 218 mg/d Group B Males: 292 mg/d Females: 251 mg/d
Salama *et al* (2010) Ain Shams, Egypt	Breast-fed rachitic infants (n=32) with 25(OH)D < 25nmol/L 17 infants with dark skin; 9 infants presented with hypocalcaemic seizures. Mean age of children who presented: With seizures: 3.67 m ± 1.6 Without seizures: 12.35 m ± 4.3 (p=0.001)	Clinical presentation, biochemical results and radiological findings (wrists, knees and ankles).	Children presenting with seizures: 25(OH)D: 24.3 ± 14.8/Ca: 65 ± 14.8 Children presenting without seizures: 25(OH)D: 43.5 ± 20.6/Ca : 85±15 25(OH)D & Ca significantly lower in children with seizure	Ca intakes not reported
Perez-Rossello *et al* (2011) Boston, USA	Children (n=40) aged 8–24 m with 25(OH)D <50 nmol/L. Identified from prospective sample examined for routine clinical care.	Radiographs from wrists & knees scored for rachitic changes on 10-pt Thacher score (and 5-pt demineralisation scale). 2 children identified as rachitic.	Children (n=2) with rachitic changes 25(OH)D: 9/Ca: 103 Children (n=34) without rachitic changes 25(OH)D: 17/Ca :104 (n=4 children could not be categorised.)	Ca intakes not reported
Munns *et al* (2012) Australia (national)	Children (n=398) with vitamin D deficiency rickets (25OHD ≤ 50 nmol/L & alkaline phosphatase >229 IU/L) and/or radiological rickets. Median age, 6.3 y (range, 0.2–15 years). 98% dark/intermediate skin colour; 18% girls partially/completely veiled. 63% born in Africa.	95 children had wrist x-rays - 71% had rachitic changes.	25(OH)D - median (range): 28 (5–50) Ca - median (range): 95.2 (48.8–117.6)	Ca intakes not reported. Rickets presentation showed seasonal variation: 60% cases identified in winter & spring, compared with 40% in summer & autumn.

Table 3: Before and after studies on rickets

Study/country	Population	Rickets diagnosis	Treatment	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Calcium intakes/ other comments
Elzouki *et al* (1989) Benghazi, Libya.	African children (n=22) with confirmed rickets admitted for treatment. Median age, 15 m (3-24 m)	Diagnosis of rickets left to individual physician. Radiograph of wrist obtained.	Daily sun exposure of 1-3 hours. After 1-2 wks sun exposure (median 9 d), given 1 dose of intramuscular D2 (15,000 µg).	At diagnosis: 50% with 25(OH)D < 20 (4-65) Ca concentrations not specified.	Ca intakes not reported.
Garabedian *et al* (1983) Paris, France	Infants & children with rickets (n=20) Age (range): 15 patients aged 4-26 months 5 patients aged 4-12 y	1 patient admitted to hospital because of overt signs of rickets; in others, rickets discovered during evaluation of pathological states	50 µg/d D2 (n=9) Single dose of 25(OH)D – 10 µg/kg (n=5) Treatment continued until rickets healed. 3 patients given Ca infusion	Pre treatment 25(OH)D: 10.1 (4.6) Ca: 75 (11) Post treatment 25(OH)D: 131.83 (44.1) Ca: 99.5 (6.4)	Ca intakes not reported.
Bhimma *et al* (1995) Durban, SA.	Black children (n=23) admitted to hospital with rickets; 14 with Ca deficiency; 9 with vitamin D deficiency Mean age = 6.1 y(2-12y);	Diagnosed clinically, radiologically & biochemically. Vitamin D deficient rickets diagnosis on basis of 25(OH)D < 25 nmol/L + other features of calciopaenic rickets. Dietary Ca deficiency considered in children with evidence of calciopaenic rickets, but normal 25OHD. Hypophosphatemic VDR rickets diagnosed by normal Ca & vit D concentrations and low PO.	125- 250 µg D3 plus 500-1000 mg calcium	<u>Vitamin D deficient patients</u> 25(OH)D: 9.25 (8.75) Ca: 83.6 (10.8) <u>Ca deficient patients</u> 25(OH)D: 45.5 (10) Ca: 86.4 (11.2)	Ca intakes not reported.
Soliman *et al* (2008) Doha, Qatar	All infants and children up to 3y attending growth clinic (n=46) between Oct 2003 and Sept 2005. Mean age = 13.1 m ± 1.1.	Clinical manifestations of rickets with serum 25(OH)D < 25 nmol/L. Radiological confirmation of rickets at the distal, ulnar or femoral epiphysis.	6 months (or more) of 7500 µg intramuscular vitamin D3.	Before treatment: 25(OH)D: 11.25 (1.4) Ca: 82.8 (10) After treatment: 25(OH)D: 111.25 (9.25) Ca: 97.6 (8)	Ca intakes not reported.
Soliman *et al* (2009) Doha, Qatar	Children(n=40) with vitamin D deficiency rickets Mean age: <u>16.1 m</u> ± 4.5	Clinical manifestations of rickets with serum 25(OH)D < 25 nmol/L. Radiological confirmation of rickets at the distal, ulnar or femoral epiphysis	Vitamin D3 250 µg/kg (max 3750 µg)	Before treatment: 25(OH)D: 15.6 (12.3) Ca: 87.6 (7.6) After treatment: 25(OH)D: 70.5 (21.8) Ca: 96 (5.6)	Ca intakes not reported.

Study/country	Population	Rickets diagnosis	Treatment	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Calcium intakes/ other comments
Ozkan *et al* (2009) Turkey	Children diagnosed as VDDR (n=21). Age: 2-24 m (median age at referral, 7.3 (2-16) m).	Clinical evidence, 2 or more of following: craniotabes (in infants > 2m), bilateral widened wrists, frontal bossing, bowing of legs, pathologic fractures, hypocalcemic tetany, hypocalcemic convulsions, Harrison's sulcus. Radiological evidence from wrist included 2 or more of following: generalized osteopenia, fraying & cupping of distal ends of radius or ulna.	All treated with intramuscular single dose of vitamin D3 (7500 µg) and oral dose of Ca (50 mg/kg/d for 10 days).	Pre treatment 25(OH)D: 10.5 (4.6) Ca: 58.2 (5.9) Post treatment 25(OH)D: 131.8 (44.1) Ca: 99.5 (6.4)	Ca intakes not reported
Thacher *et al* (2009) Jos, Nigeria.	Prepubertal children (n=17) with clinical signs of rickets. Mean age(range):44.5 m (28–118)	Subjects required radiological score of at least 1.5 on a previously validated 10-point scale for assessing severity of childhood rickets (Thacher et al, 2000)	Maize porridge (150ml) & orange juice (50ml) with added Ca (120 mg calcium glubionate & 20g calcium chloride). After drawing blood, 0.5 mg Ca infused slowly. After 4 days, single oral dose of vitamin D2 or D3 (1250 µg).	<u>Baseline</u>**:** 25(OH)D: 50 (12.5–80) Ca: 88 ± 10	Ca intakes (mg): 182 (73)
Cesur *et al* (2011) Van, Turkey	Retrospective study of medical records of patients (n=946) with nutritional rickets (25(OH)D <25 nmol/L; clinical or radiological signs of rickets). Age 0-16y: 0-23 m: 51.4% 24 m – 5.9y: 9.2% 6–11.9 y: 17.3% 12-15 y: 22.1%	Clinical signs: Craniotabes, epiphyseal swelling, rachitic rosary, Harrison groove or hypocalcaemic seizures. Radiological evidence: Widening of growth plates with irregularity and cupping of metaphyseal borders.	1) 3750 µg (single dose); 2) 7500 µg (single dose); 3) 40 µg/d for 5- 6 months; 4) 110 µg/d for 3 months.	<u>Baseline</u> 25(OH)D: 15 (post-treatment not reported) <u>Ca before/after treatment:</u> 0-23 m: 86/87 24m-5.9y: 86/86 6-11.9y: 87/93 12-15y: 88/91	Ca intakes not reported.

Table 4: Case control studies on rickets

Study/Country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Calcium intakes/other comments
Arnaud *et al* (1976) North Midwestern USA & Canada &	Children with nutritional deficiency rickets[113] (n=9) Age: 2- 42 m Rachitic control infants (n=6) with familial hypophosphataemic rickets Age-matched control infants (n=9; outpatients with problems unrelated to skeletal disease).	Diagnosis of VDDR confirmed with healing of bone lesions & normalisation of serum Ca, P and alkaline phosphatase in response to vitamin D supplementation (125 µg/d for 4 weeks). Rachitic control patients: diagnosis through family history (n=5) & failure to respond to vitamin D supplementation (125 µg/d).	25(OH)D Mild deficiency (n=2): 45 (7.5) Moderate (n=5): 30 (5) Severe (n=1): 20 Controls (n=9): 90 (30) Rachitic controls (n=6): 90 (37.5) Ca Moderate deficiency: 96 (6) Controls: 101 (4) Rachitic control mean: 99 (2)	Ca intake not reported. Specimens from patients with mild rickets obtained mid-winter; from patients with moderate & severe rickets, obtained when more sun exposure possible.
Oginni *et al* (1996) Ile-Ife, Nigeria	Children with active rickets (n=26) Age 1-5 y Healthy controls from the same age and community group (n=90).	Clinical criteria included swollen wrists, rachitic rosaries and angular deformities of the knees, widening and cupping of the metaphysis, fraying and thickening of the physis and generalized rarefaction.	Cases 25(OH)D: 36 ± 28/Ca: 82.4 ± 9.2 Controls 25(OH)D: 69 (22)/Ca: 94 (9.2) 25(OH)D & Ca significantly lower in cases	Ca intake not reported.
Majid Molla *et al* (2000) Kuwait	Infants with clinical features of rickets (n=103) Mean age: 14.5 m (5.2) Age and sex matched controls (n=102). Mean age 15.2 m (6.28)	Clinical criteria: hypotonia, skeletal deformity (bowing of the legs, deformity of end of the long bones), rachitic rosary, delayed closure of the fontanel, delayed walking and delayed dentition.	Cases 25(OH)D: 26.5 (15.5)/Ca: 89.6 (11.2) Controls 25(OH)D: 83.5 (74.8)/Ca: 98 (6) 25(OH)D & Ca significantly lower in cases	Ca intake not reported.
Thacher *et al* (2000) Jos, Nigeria	Children with symptoms of rickets (n=123). Age: 1-14y Age, weight and sex-matched controls (n=123) from same community with no sign of rickets.	Evidence of bone deformity: knock knees, bow legs or enlarged wrists.	Means (SD) or median (25th and 75th centiles). Cases 25(OH)D: 32 (22-40)/Ca: 77.2 (8.8) Controls 25(OH)D: 50 (42-62)/Ca: 89.6 (6) 25(OH)D & Ca significantly lower in cases	Calcium intake (mg): Cases: 217 (88) Controls: 214 (77) Sun exposure was not significantly different.
Al-Mustafa *et al* (2007) Al-Khobar and Dammam, Saudi Arabia	Children clinically diagnosed with rickets (n=61). Mean age: 14.8 m Controls – age & sex matched children (n=58) without clinical rickets attending hospital for other blood investigations. Mean age: 16.5 m	Clinical examination, biochemical data & radiological manifestation of active disease.	Cases 25(OH)D: 75% < 20; 25% >20 Ca: 31% < 21.25[114]; 59% 21.25–26.25; 10% >26.25 Controls 25(OH)D:26% < 20; 74% > 20 Ca: 7% < 21.25; 78% 21.25–2.63; 15% >26.25	Calcium intakes not reported Recruited from March 2004 to February 2005

[113] Classified as having mild, moderate or severe rickets according to the criteria of Fraser et al (1967).
[114] Ca concentrations < 21.25 mg/L defined as hypocalcaemic.

Study/Country	Population	Rickets diagnosis	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Calcium intakes/other comments
Prentice *et al* (2008) MRC Keneba and MRC Fajara in The Gambia.	Analysis of stored samples from children (n=46) who had presented with rickets-like bone deformities. Mean age (-1SD, +1SD) = 3.4 (1.9, 6.4) y Biochemical data from earlier study of local children (n=147) used as reference. Mean age: 4.0 (3.0, 5.3)	Radiographs reviewed and assessed by Thacher et al (2000) method. Patients classified as having active rickets (bone deformities plus biochemical indicators) or non-active rickets (bone deformities only).	Active rickets (n=13): 25(OH)D: 42.4 (28.6, 56.1)/Ca: 91 (84, 98.4) Non-active rickets (n=33) 25(OH)D: 50.7 (37.9, 63.4)/Ca: 95 (89.6,100.4) Reference children (n=147) 25(OH)D: 95.0 (75.3, 114.6)/Ca: 92 (84.4, 99.6)	<u>Ca intakes</u> Majority prescribed Ca following presentation at clinic. Median dose over 6 months: 117 mg/d.
Ozkan *et al* (2009) Erzurum, Turkey	Cases: children attending paediatric outpatient clinic (n=39). Mean age: 10±7.5 m Controls: age-matched children (n=28) in outpatient clinic with minor illness & serum 25(OH)D > 25nmol/L.)	Identified through clinical signs & confirmed through radiological & laboratory examination. Clinical rickets diagnosed if 2 or more of following criteria present: craniotabes, rachitic rosary, bilateral widened wrists, frontal bossing, bowing legs, fractures, tetany, convulsion and Harrison's sulcus.	<u>Cases</u> (n=39) 25(OH)D:14.5±7/Ca: 75±19 Controls (n=15) 25(OH)D:44.7±16.5/Ca: 95±90	Ca intakes not reported. Mean 25(OH)D concentration in cases diagnosed in summer months was higher than in those diagnosed in winter months.

Table 5: Intervention studies on rickets

Study/Country	Population	Rickets diagnosis	Treatment	Mean 25(OH)D (nmol/L) & Ca (mg/L) concentrations	Ca intakes/other comments
Cesur *et al* (2003) Van, Turkey	Patients (n=56) with nutritional VDDR Mean age 10.7 (6.1) m Age-matched controls (n=20). Mean age: 11.7 (8.0) m	Based on history, clinical and laboratory findings plus left wrist x-ray. Stage of rickets determined according to serum CA, P & ALP concentrations Stage 1: serum Ca low, P normal Stage 2: serum Ca normal due to compensatory hyperparathyroidism Stage 3: both Ca & P low.	Participants randomly assigned to vitamin D (single dose): Gp 1 (n=20): 3750 µg Gp 2 (n=20): 7500 µg Gp 3 (n=16): 15000 µg	Patient group before treatment**:** 25(OH)D Stage 1: 15.8 (6.4) Stage 2: 15.4 (4.8) Stage 3: 14.7 (3.9) Ca: 78.2 (13.5) Control group Ca: 89.0 (4.6)	Ca intakes not reported.
Rajah *et al* (2008) Abu Dhabi, UAE	Retrospective study of children (n=31) with nutritional rickets. Mean age: 17.3 (6.3) m	Clinical & x-ray features of rickets & elevated ALP. Clinical features: widened wrists, frontal bossing, bowing of legs and costochondral thickenng..	Treated with 50-125 µg/d of D2 drops for 3 months and subsequently 10 µg/d for a variable period.	Baseline: 25(OH)D: 30.8 (18.7) Ca: 91 (8))	Ca intakes not reported.
Emel *et al* (2012) Bezmialem, Turkey	Children (n=42) diagnosed with VDDR between October 2010-March 2011. Age: 5m- 3 y	Clinical features of all patients: enlarged wrist & ankles (95%), failure to thrive (76%), muscle weakness (52%), bowing of tibia & femur (42%), frontal bossing (28%), delayed fontanel closure (14%), leg pain (14%), rachitic rosary (11%), Harrison grove (11%), seizures (2%).	Group 1: 50 µg/d D3 for 6 wks Group 2: 3,750 µg single dose D3 Both groups received calcium lactate (50mg/kg/day) for first 2 wks of intervention.	25(OH)D (baseline) Group 1: 35.9 (12.7) Group 2: 34.2 (12.2) Calcium (baseline) Group 1: 99 (7) Group 2: 92 (7)	Ca intake not reported. All received calcium lactate (50mg/kg/d) for 2 wks.

Table 6: Observational studies on osteomalacia

Study/Country	Population	Osteomalacia diagnosis	Mean 25(OH)D (nmol/L) & Ca (mmol/L) concentrations
Preece *et al* (1975) Glasgow, Scotland	Patients (n=35) of South Asian ethnic origin with overt rickets or osteomalacia 62% hypocalcaemic (Ca <88mg/L) Age not reported.	Clinical and biochemical evidence. Radiological confirmation in majority.	Those with hypocalcaemia (n=22) 25(OH)D: 7.5 Others (n=21) 25(OH)D: undetectable
Gifre *et al* (2011) Barcelona, Spain	Adults (n=28); mean age 55±28 y 26 white; 2 south Asian 14/28 hypo-phosphatasia osteomalacia 13/28 vitamin D osteomalacia	Bone biopsy and/or by the Bingham & Fitzpatrick criteria defined as two of the following: low Ca, low P, elevated total AP, suggestive radiographs	Whole group: 25(OH)D: 40 (37)/Ca: 2.2±0.3 Vitamin D osteomalacia patients: 25(OH)D: 15 (5)/Ca: 1.98 ± 0.33 Hypo-phosphatasia group: 25(OH)D: 62 (42)/Ca: 2.25 ± 0.18
Torun *et al* (2013) Istanbul, Turkey	Patients (n=543); aged 1-17 y. Referred to hospital with symptoms of rickets, osteomalacia or vitamin D deficiency.	Classified as vitamin D deficient or insufficient based on 25(OH)D concentrations: Vitamin D deficient: <25 nmol/L Vitamin D insufficient: 25- 50 nmol/L	1-3 y: 25(OH)D: 32 (11)/Ca: 98 (10) 4-6 y: 25(OH)D: 28 (11)/Ca: 101 (10) 7-11 y 25(OH)D: 23 (11)/Ca: 96 (5) 12-17 y: 25(OH)D: 20 (11.5)/Ca: 94 (8)

Table 7: Case reports of osteomalacia

Study/Country	Population	Osteomalacia diagnosis	Mean serum 25(OH)D (nmol/L) & Ca (mg/L) concentrations
Clark *et al* (1972) Newcastle upon Tyne, UK	Patients: n=15; age 11-42y. South Asian, living in the UK ≥ 1y (mean 4.5 y). Presented with spontaneous pain, usually in legs, 4 seen following minor injury Blood relatives: n=18, age 8-48 y; 3 with symptoms of limb pain.	Biochemical evidence of rickets and osteomalacia. 12/15 patients and 2/18 relatives had radiological evidence. 2/3 patients histological evidence.	25(OH)D: not reported Ca Patients: 84 Blood relatives: 92
Moncrieff & Fadahunsi (1974) Derby, UK	Female (pregnant); age 30 y. South Asian ethnic origin. Presented with pain in sacrum & waddling gait during latter part of pregnancy	Biochemical and clinical evidence of osteomalacia; x-ray of pelvis did not show osteomalacia.	*Mother (after delivery)* 25(OH)D: 17.7/Ca: 82
Russell & Hill (1974) Manchester, UK	Female (pregnant) - age 30 y; South Asian ethnic origin Presented with signs of pre-eclampsia at 37 weeks gestation (not treated) Infant - male, delivered spontaneously	No clinical evidence of osteomalacia at 37 weeks gestation; x-ray confirmed fetal rickets. *4 months after delivery*: biochemical evidence of osteomalacia but x-ray normal Infant – wrist x-ray confirmed rickets.	*At 37 weeks gestation*: 25(OH)D : 7.5/Ca: 72 *4 months after delivery:* 25(OH)D: 6.7/Ca: 76
De Torrente de la Jara *et al (*2004) Switzerland	Female asylum seekers (n=11) from Bosnia, Afghanistan, Somalia, Albania, Ethiopia Presented with minimal exposure to sunlight & history of bone pain, proximal muscular weakness, change in gait, or fatigue.	Measurement of 25(OH)D concentration.	25(OH)D: 10.9 (3.8) Ca: 87.6 (3.6)
Cardinal & Gregory (2009) Cambridge, UK	Female, age 75 y. White Schizophrenic, inpatient in psychiatric hospital for > 35 years; investigated due to persistent elevated alkaline phosphatase & intermittently low phosphate and calcium concentrations.	Biochemical evidence of osteomalacia	25(OH)D: 10.5
Thabit *et al* (2011) Ireland	Case 1: Male, age 31 y. S Asian ethnic origin, living in Ireland (5y). 2 day history of upper & lower limb tetany; unable to stand unaided due to progression of bilateral lower limb weakness. Case 2: Female, age 34 y. S Asian ethnic origin, living in Ireland (9 y). Pains pelvic region for 4y; proximal muscle weakness & difficulty walking, significant proximal myopathy & waddling gait.	Biochemical evidence.	Case 1 25(OH)D: 5.5/Ca: 57.2 Case 2 25(OH)D: 16/Ca: 81.6
Mittal *et al* (2012) India	Male, age 41 y. Presented with gradually progressive quadriparesis over previous 6 m.	Based on symptoms.	25(OH)D: 20.3 Ca: 81.2

Table 8: RCTs on effect of vitamin D supplementation during pregnancy on bone health indices in the offspring

Author/Year	Population	Intervention/Duration	Mean (SD) maternal 25(OH)D concentration (nmol/L) (SD)	Mean infant BMC (g)/ bone area (cm^2)/ BMD (g/cm^2) measured within 2 weeks of birth	Effect on markers of bone health in infants
Cooper *et al* (2016) Southampton, Oxford & Sheffield, UK	Pregnant women (n=1134) with serum 25(OH)D 25-100 nmol/L & serum Ca <2.75 mmol/L at 10-17 weeks' gestation & their infants Mean age: 30.5 (5.2) y	Randomly assigned at 14 weeks gestation to: 1. 25µg D3 2. placebo (All participants were able to continue self-administration of antenatal vitamins containing ≤10µg/d vitamin D) Duration: until delivery	<u>Baseline</u> 1. 46.7 (17.7) 2. 45.9 (17.0) (difference: p>0.05) <u>34 weeks gestation</u> 1. 67.8 (22.1) 2. 43.3 (22.3) (difference: p<0.0001)	<u>BMC</u> 1. 61.6 (95%CI, 60.3-62.8) 2. 60.5 (95%CI, 59.3-61.7) <u>Bone area</u> 1. 301.6 (95%CI, 297.8-305.4) 2. 297.8 (95%CI, 293.7-301.9) <u>BMD</u> 1. 0.203 (95%CI, 0.200-0.205) 2. 0.203 (95%CI, 0.200-0.205)	No difference between intervention & placebo group in BCM (p = 0.21), bone area (p = 0.18) or BMD (p = 0.96). BMC significantly greater in intervention group for infants born in winter (December-February) (MD 5.5g; 95% CI, 1.8-9.1; p = 0.04). Similar winter-birth effect observed for whole body bone area (MD 11.5 cm^2; 95% CI, 0.1-22.9; p = 0.05) & BMD (MD 0.01 g/cm^2; 95% CI, 0.00-0.02; p = 0.04).

Table 9: Cohort studies on association between maternal 25(OH)D concentration and bone health indices in the offspring

Author/Year	Population	Design	Mean maternal 25(OH)D concentration (nmol/L)	Mean cord 25(OH)D concentration (nmol/L)	Findings
Mahon *et al* (2010) Southampton, UK	Pregnant women (n=424) Mean age: 31.3 (3.6)y	Longitudinal	At 34 wks: Median: 31.3 (IQR: 41-85) 5.9% < 25 30.7% < 50	Not measured	Infants born to mothers with 25(OH)D concentration < 50 nmol/L had increased femoral splaying indices compared with > 50 nmol/L.
Prentice *et al* (2009) The Gambia, Africa	Pregnant women (n=125) and their infants Mean age: 27.4 (7.5)y	Original Ca RCT. Cross-sectional with longitudinal follow up	At 20 wks: 103 (25) At 36 wks: 111 (27)	Not measured	No relationship between maternal 25(OH)D concentrations and BMC/BMD of radius and whole body in infants.
Viljakainen *et al* (2010) Helsinki, Finland	Pregnant women (n=125) Mean age:31 (4) y	Cross-sectional and longitudinal	1st trimester: 41 (13.6) Postpartum: 45.1 (11.9)	50.7 (14.9)	Median 25(OH)D concentration (42.6 nmol/L) used as cut off. Tibia BMC of infants born to mothers above the median was 0.047g/cm higher than those born to below median group but there was no difference in BMD.
Young *et al* (2012) Baltimore, USA	171 Pregnant adolescent girls (n=171) and their infants Age: <18 y	Longitudinal follow up	26 weeks gestation and at delivery: 54.7 (27.5)	Not measured.	Maternal 25(OH)D > 50 nmol/L significantly positively associated with fetal femur and humerus z scores. ($p < 0.01$).
Dror *et al* (2012) California, USA	Mother-infant pairs (n=80) African Americans (n=40); mean age: 25.4 (5.2) Non African-Americans (n=40); mean age 28.3 (5.6)	Cross-sectional with longitudinal follow up	African American: 69.1 (26.1) Non-African American: 82.3 (30.3)	African American: 36.0 (31.7, 40.8)* Non-African American: 48.2 (41.4, 56.1)*	No association between maternal or cord serum 25(OH)D concentration and infant whole body BMC.

*Geometric means & 95% confidence intervals presented for variables requiring log transformation.

Table 10: RCTs on effect of vitamin D supplementation on bone health indices in infants

Study/Country	Population	Intervention & study duration	Mean 25(OH)D conc (nmol/L)	Bone health results	Comments
Kim *et al* (2010) Cheongiu, Korea	New born breast fed infants (n=74)	3 groups 1. Formula 2. 10 µg/d* 3. No intervention 12 months	At birth: 44.2 (17.4) After 12 m: 1. 121.8 (24.3) 2. 106.8 (24.5) 3. 82.9 919.7)	BMD significantly higher in formula fed group than vit D intervention & no intervention groups. BMC significantly higher in in formula fed group than vit D group but no differences between formula-fed group and no intervention group or between vit D group and no intervention group.	Poor study design. Number of core uncertainties.
Kumar *et al* (2011) New Delhi, India	Low birth-weight newborn infants aged 7 days (n=2079)	35µg/wk or placebo. 6 months	At baseline: Not reported. After 6 m: Vitamin D: 55 (22.5) Control: 36 (25.5)	Vit D supplementation increased SD z scores at 6m for weight, length and arm circumference and significantly decreased head circumference.	Number of participants less than highlighted in abstract (n=216, vit D group; n=237, placebo group).
Abrams *et al* (2012) Houston, USA	Infants aged 1 wk (n=49)	10 µg/d 3 months	At baseline: Non-Hispanic: 55.7 (23.5) Hispanic: 40.9 (16.2) After 3m White - 57.4 (23.5)) Hispanic: 42.2 (18)	No relationship between cord serum 25(OH)D and BMC or BMD during 1st week of life or after 3 months of vitamin D supplementation.	Small study. Limited time allowed to show effect on bone indices.
Holmlund-Suila *et al* (2012) Finland	Infants aged 2 wks (n=113)	1. 10 µg/d 2. 30 µg/d 3. 40 µg/d 10 weeks	1. 88 2. 124 3. 153	No difference in PTH or bone turnover markers. Using peripheral quantitative computed tomography in a multivariate ANCOVA there was a trend toward better stress and strain index and larger total bone and cortical bone area was noted with higher vitamin D doses.	10 weeks short treatment time to show change in bone architecture and mineral accrual.

*Supplement also contained contains vitamin A (1,500 IU/mL), vitamin E (5 IU/mL), vitamin C (35 mg/mL), thiamin (0.5 mg/mL), riboflavin (0.6 mg/mL), niacin (8 mg/mL), vitamin B6 (0.4mg/mL), iron (10 mg/mL), and fluoride (0.25 mg/mL).

Table 11: Systematic review of RCTs on effect of vitamin D supplementation on bone health indices in children and adolescents

Author	Methods	Results	Conclusions
Winzenberg *et al* (2011) Vitamin D supplementation for improving bone mineral density in children.	**Selection criteria:** *Inclusion:* RCTs of vitamin D supplementation compared with placebo; treatment period of ≥ 3 m; trials in children and adolescents (aged < 20 y) without coexistent medical conditions or treatments causing osteoporosis; trials of vitamin D supplementation regardless of type or dose of vitamin D supplement or method of administration, compared with placebo. *Exclusion:* Studies performed exclusively in neonates (aged < 1 m); trials with treatment period <3 m. Outcome measure BMD	6 RCTs (n=884; age, 8-17y; vitamin D3 dose, 3.3 µg/d to 350 µg/wk; mean baseline serum 25(OH)D - 17.7-49.5 nmol/L) 2 studies included co-interventions (one of milk providing 245 mg/d of Ca to both vit D and control groups and one of 1000 mg/d $CaCO_3$ to both vit D and control groups. ***Results*** Total body BMC (5 RCTs): SMD 0.10 (95%CI -0.06, 0.26) (p=0.21) Hip BMD (4 RCTs): SMD 0.06 (95%CI -0.18, 0.29) (p=0.64) Forearm BMD (3 studies): SMD 0.04 (95%CI -0.36, 0.45) (p=0.84) Lumbar spine BMD (5 studies): SMD 0.15 (95%CI -0.01, 0.31) (p=0.07) *Comparison by baseline 25(OH)D concentration (<35 and ≥35 nmol/L)* Total body BMC >35nmol/l (2 RCTs): SMD -0.07 (95%CI 0.33, 0.18) (p=0.57) <35nmol/l (3 RCTs): SMD 0.21 (95%CI 0.01, 0.41) (p=0.04) Hip BMD >35nmol/l (3 RCTs): SMD -0.02 (95%CI -0.31, 0.28) (p=0.91) <35nmol/l (1 RCT): SMD 0.25 (95%CI -0.07, 0.58) (p=0.12) Lumbar spine >35nmol/l (3 RCTs): SMD 0.09 (95%CI -0.10, 0.28) (p=0.35) <35nmol/l (2 RCTs): SMD 0.31 (95%CI 0, 0.61) (p=0.048) Forearm >35nmol/l (2 RCTs): SMD 0.12 (95%CI -0.62, 0.85) (p=0.76) <35nmol/l (1 RCT): SMD -0.06 (95%CI -0.38, 0.26) (p=0.71)	Vitamin D supplementation had no statistically significant effects on total body BMC, hip bone BMD or forearm BMD. Trend to small effect on lumbar spine BMD (p=0.07) In studies with baseline 25(OH)D <35 nmol/L effect of vitamin D supplementation on total body BMC was significant (p-0.04) and bordering on significance for lumbar spine BMD (p=0.05).

Table 12: Intervention studies on effect of vitamin D supplementation on bone health indices in children and adolescents

Author/Year	Population	Intervention/ Duration	Mean baseline 25(OH)D concentration (nmol/L)	Mean post intervention 25(OH)D conc (nmol/L)	Effects on bone health indices
Park *et al* (2010) Purdue, USA	Females (n=11) Mean age: 13.2y	25ug/d D3 4 wks	46.8 (±4.1)	60.1 (±3.3)	Increased urine Ca excretion but did not increase fractional Ca absorption, net Ca absorption or Ca retention.
Molgaard *et al* (2010) Copenhagen & Frederiksbar, Denmark	Females (n=221) Mean age: 11.4y	1. 5 ug/d D3 2. 10 ug/d D3 3. Placebo 12 months	1. 41.9 (±17.6) 2. 44.4 (±16.6) 3. 43.4 (±17.1)	1. 52.9 (±16.3) 2. 57.9 (±14.3) 3. 39.7 (±17.7)	No effect of either 5 or 10 µg/d vitamin D on bone markers of turnover or whole body/lumbar spine bone mineral augmentation. Vitamin D increased whole body BMD (p=0.007) & BMC (p=0.048) in FF VDR genotype but not in Ff or ff VDR genotypes.
Ghazi *et al* (2010) Tehran, Iran	Males (n=105) & females (n=105) Mean age: 16.3y	1. 1250 µg/month D3 (≡ 40 µg/d) 2. 1250 µg/bi-monthly D3 (≡ 20 µg/d) 3. placebo 6 months	32 [±22] 28.3 (±14.5) 29 (±18)	1. 60 (±27.5) 2. 45.8 (±24) 3. 29 (±17.5)	No change in urinary CTX. Significant increase in osteocalcin in both vitamin D groups. Significant decrease in PTH.
Khadilkar *et al* (2010) India	Girls (n=50) Mean age: 14.6y	1. 7500 µg D2, 4 times/year + 250 mg/d Ca. 2. Placebo 4 times/y + 250 mg/d Ca 1 year	*Median* 1. 24.5 (12.7-33.2) 2. 20.8 (12.7-30.4)	*Median* 1. 75.2 (64.2-85.5) 2. 28.1 (16.7-34.0)	No significant difference in bone outcome measures in the two groups. However, positive effect of intervention in the size adjusted total body bone area (p<0.05), total body BMC (p<0.05) and lumbar spine BMC (p<0.05), and positive trend in lumbar spine bone area (p=0.07) in girls who were within 2 years of menarche. Pilot study.
Ward *et al* (2010) Manchester, UK	Postmenarchal girls (n=69) Mean age: 13.8y 88% South Asian	1. 3750 µg D2, 4 times/year 2. Placebo 4 times/year Duration: 1 year	1. 18.1nmol/l (8.0) 2. 17.9nmol/l (7.4)	1. 56.0 nmol/l (8.9) 2. 15.7nmol/l (6.6)	Effects of vitamin D supplementation on BMC/BMD were non significant at all skeletal sites.

Table 13: Intervention studies on effect of vitamin D supplementation on muscle strength and function in children and adolescents

Author/Year	Population	Intervention/Duration	Mean (SD) baseline 25(OH)D conc (nmol/L)	Mean (SD) post intervention 25(OH)D conc (nmol/L)	Effects on muscle strength and function
Ward *et al* (2010) Manchester, UK	Postmenarchal adolescent girls with 25(OH)D <37.5 nmol/L (n=69) 88% South Asian, 7% Black, 1% Middle Eastern & 3% mixed race Mean age: 13.8 y	1. 3.750 µg D2 , 4 times/y 2. Placebo 4 times/y Duration: 1 y	1. 18.1 (8.0) 2. 17.9 (7.4) (difference p > 0.05)	1. 56.0 (8.9) 2. 15.7 (6.6)	Mean baseline serum 25(OH)D concentration increased significantly in intervention group (p=<0.001). Efficiency of movement increased significantly (by 5%; p = 0.02) in the intervention group. There were no improvements in muscle force or power. An interaction was found between baseline serum 25(OH)D concentration and jump velocity in the intervention group (p=0.02); those with lower baseline concentrations had greater change in velocity than those with higher concentrations.

Adults (under 50 years)

Table 14: RCTs on effect of vitamin D supplementation on bone health indices in adults < 50y

Author/Year	Population	Intervention/ duration	Mean baseline 25(OH)D concentration (nmol/L)	Mean post intervention 25(OH)D concentration (nmol/L)	Effects on bone health indices
Islam *et al* (2010) Dhaka, Bangladesh	Pre-menopausal women (n=200) Age: 8-36 y	1. 10 µg/d 2. 10 µg/d & 600 mg calcium 3. 10 µg/d & 600 mg calcium & multiple mineral supplement 4. Placebo Duration: 1 year	1. 37.1 (12.1) 2. 37.8 (10.9) 3. 36.9 (12.5) 4. 35 (9.4)	Increased by: 1. 32.2 (23.6) 2. 32.4 (24.3) 3. 27.9 (24.8) 4. 0.5 (13.8)	Significant increase in BMD & BMC at femoral neck in supplemented groups compared to placebo ($p < 0.001$) Significant increase in BMD & BMC of greater trochanter and Ward's triangle compared with the placebo group ($p < 0.05$). No significant differences in BMD and BMC of the lumbar spine.

Table 15: Meta-analyses on effects of vitamin D supplementation on muscle strength in adults < 50y

Study	Methods	Results	Conclusions
Tomlinson *et al* (2014)	Selection criteria *Inclusion:* publication in English, inclusion of control group, muscle strength measured as one of the primary outcomes; at least one arm of study included supplementation of participants with vitamin D only. *Exclusion:* Aged < 18 y; non-healthy participants. Outcome measure Upper and lower limb muscle strength.	Included trials 6 RCTs, 1 controlled trial (n=310 adults; mean age 24.1y (range 21.5-31.5y). Vitamin D supplement dose range: 50 µg/d to 1500 µg/wk. 6 trials administered D3; 1 trial did not specify vitamin D type. Outcome measures *Upper limb strength* Vitamin D supplementation had significant effect SMD= 0.32 (95% CI, 0.10-0.54) ($p = 0.005$) *Lower limb strength* Vitamin D supplementation had significant effect SMD= 0.32 (95% CI, 0.01-0.63) ($p = 0.04$))	Vitamin D supplementation improves upper and lower limb muscle strength in adults under 40y.

Table 16: RCTs on effects of vitamin D supplementation on stress fracture reduction in adults < 50y

Study/country	Population	Intervention & duration	Mean baseline & post intervention 25(OH)D (nmol/L)	Results
Lappe *et al* (2008) USA	Female Navy recruits (n=5201) Median age 19 y (17-35y)	1. 20 µg vitamin D & 2000 mg Ca 2. Placebo Duration: 8 weeks	Not reported.	Supplemented group had 20% lower incidence of stress fracture than control group (5.3% vs 6.6%) RR = 0.80 (95% CI, 0.64-0.99) (p < 0.0026) Per protocol analysis: 21% fewer fractures in supplemented vs control gp (6.8% vs 8.6%).

Table 17: Meta-analysis of observational studies on association between serum 25(OH)D concentration and stress fractures in adults < 50y

Study	Methods	Results	Conclusions
Dao *et al* (2014) Serum 25-Hydroxyvitamin D levels and stress fractures in military personnel: a systematic review and meta-analysis	Selection criteria: *Inclusion:* Any type of study examining association between stress fractures and serum 25(OH)D concentration; participants aged ≥ 18 y; participants involved in any branch of a country's military force (i.e. army, navy, air force, marine corps); and studies published in the English. *Exclusion:* Case reports and series (< 10 participants); review articles; guidelines; basic science and animal studies; conference abstracts; studies of athletes. Outcome measure Stress fractures	8 studies (n=2634; age, 18-30y): 5 prospective cohort studies, 2 nested case-control studies; 1 case-control study. Overall mean serum 25(OH)D concentration significantly lower for stress fracture cases than controls: MD, -6.1 nmol/L (95% CI, -10.1 to -2.1; p = 0.003) with between study heterogeneity (I^2=53%). *At time of diagnosis (3 case-control studies):* 25(OH)D concentration significantly lower in stress fracture cases compared with controls: MD, -5.6 nmol/L (95% CI, -9.7 to -1.6; p=0.007) with moderate between study heterogeneity (I^2 = 42%) *At time of entry to basic training (5 studies):* Pooled MD of serum 25(OH)D between stress fracture cases and controls: -6.6 nmol/L (95% CI, -14.5 to 1.3; p = 0.10) (I^2 = 65%)	Results suggest some association between serum 25(OH)D concentration and lower extremity stress fractures in military personnel.

Table 18: Cohort studies (not included in above meta-analysis) on association between serum 25(OH)D concentration and stress fractures in adults < 50y

Study/Country	Population	Follow-up	Mean baseline 25(OH)D concentration (nmol/L) (SD)		Results	Comments
Davey *et al* (2016) UK	Male RM recruits on a RM training programme (n=1,082) Age (mean): 20 y	32 weeks	With fracture: Without fracture :	64.2 (28.2) 69.6 (29.3)	25(OH)D < 50 nmol/L v > 50 nmol/L) OR = 1.6 (95% CI, 1.0-2.6)	Further studies into the effects of stress fracture risk are warranted.

Adults (50 years and above)

Table 19: Meta-analysis of RCTs on effects of vitamin D supplementation on bone health indices in adults ≥ 50y

Study	Methods	Results	Conclusions
Reid *et al* (2014) Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis	Selection criteria: *Inclusion*: RCTs comparing interventions that differed only in vitamin D content; in adults aged > 20 y; intervention with vitamin D2 and D3 but not vitamin D metabolite. Any other intervention (e.g. calcium) had to be same in all groups. *Exclusion*: studies of individuals with other disorders likely to affect bone and calcium metabolism. Outcome measure: BMD	23 trials (n=4082, mean age 59y, mean duration 23.5 months) Mean 25(OH)D < 30 nmol/L in 5 studies, 30–50 nmol/L in 3 studies, 50–75 nmol/L in 11 studies, and > 75 nmol/L in 1 study. Ca supplements administered to all participants in 12 trials. Weighted mean difference (%) (95% CI) in BMD *Lumbar spine* (17 RCTs): 0.0 (-0.2 to 0.3); p = 0.8 *Femoral neck* (13 RCTs): 0.8 (0.2 to 1.4); (p=0.005) *Hip/trochanter* (15 RCTs): 0.2 (-0.1 to 0.4); (p=0.17) *Total body* (8 RCTs): -0.3 (-0.7 to 0.1); (p=0.2) *Forearm* (6 RCTs): -0.3 (-0.7 to 0.1); (p=0.09)	Small benefit at femoral neck but no effect at any other site.

Table 20: RCTs (not included in above meta-analysis) on effect of vitamin D supplementation on bone health indices in adults ≥ 50y

Study/country	Population	Intervention & Duration	Mean baseline 25(OH)D (nmol/L)	Mean post intervention 25(OH)D (nmol/L)	Results
Karkkaiinen *et al* (2010) Kuopio, Finland	Postmenopausal women (n=593) Mean age: 67.4 (2) y	1. 20 µg/d vitamin D & 1000 mg Ca 2. Placebo Duration: 3 y	1. 50.1 (± 18.8) 2. 49.2 (± 17.7)	1. 74.6 (± 21.9) 2. 2. 55.9 (± 21.8)	Significant increase in total body BMD in vit D groupp than in control group (-2.69% vs -2.83%; p=0.03). No difference between groups in BMD changes at spine, femoral neck, trochanter & total proximal femur.
Macdonald *et al* (2013) Aberdeen, Scotland	Healthy postmenopausal women (n=305) Mean age 64.6 (2.3)	1. 10 µg/d D3 2. 25 µg/d D3 3. Placebo Duration: 1 year	1. 33.4 ± 13.2l 2. 33.2 ± 13.8l 3. 35.8 ± 16.4	1. 65.0 ± 19.7 2. 75.9 ± 18.9 3. 32.0 ± 14.9	Mean BMD loss at hip significantly less for 25 µg/d vit D group (-0.05%±1.46%) compared with 10 µg/d vit D group (-0.57%±1.33) or placebo group (-0.60%±1.66). BMD change at lumbar spine not significantly different between groups.

Table 21: Cohort studies on association between 25(OH)D concentration and bone health indices in adults ≥ 50y

Study/Country	Population	Follow-up	Mean 25(OH)D (nmol/L)	Results
Ensrud *et al* (2009)	Community dwelling men (n=1279) Age: 65y +	4.4y	59.4 *Median:* 63.4 (50.9-75.1)	Lower 25(OH)D associated with higher rates of bone loss at total hip (ptrend=0.01). Majority of effect observed among men in lowest quintile (< 47.7 nmol/L) who experienced 1.5-fold higher rate of hip bone loss (p=0.003 for Q1 vs Qs 2-5). In men ≥ 75y, lower 25(OH)D associated with higher rates of hip bone loss (p trend <0.001); no association between 25(OH)D & hip bone loss rate among men < 75y.

Table 22: Meta-analyses of trials on effect of vitamin D supplementation (+/- calcium) on fracture risk in adults ≥ 50y

Study	Methods	Results	Author's conclusions
Avenell *et al* (2014)	Selection criteria: *Inclusion:* randomised or quasi-randomised trials that compared vitamin D related compounds, alone or with calcium, against placebo, no intervention or calcium alone and that reported fracture outcomes in older people. *Exclusion:* interventions that included treatments other than vitamin D or calcium. Studies in which participants were on corticosteroid therapy. Outcome measure Primary outcome: hip fracture. Secondary outcomes: any non-vertebral fracture, vertebral fracture, any new fracture, adverse effects.	53 trials (n=91,791): 31 trials on vitamin D (+/- calcium) in prevention of fractures in the community, nursing home or hospital inpatients; 22 trials examined calcitriol or alfacalcidol, mostly with participants who had osteoporosis. ***Vitamin D alone vs placebo or no treatment*** 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) Any new fracture (15 trials, n=28,271): RR=1.03 (95% CI, 0.96-1.11) ***Vitamin D plus calcium vs calcium alone*** Hip fracture (7 trials, n=7411): RR=0.84 (95% CI, 0.63-1.13) Non-vertebral fractures (6 trials, n=3336): RR=0.96 (95% CI, 0.76-1.16) Vertebral fracture (2 trials, n=2681): RR=0.14 (95% CI, 0.01-2.77) ***Vitamin D plus calcium vs placebo or no treatment*** Hip fracture (9 trials, n= 49,853): RR=0.84 (95% CI, 0.74-0.96) Non-vertebral fractures (8 trials, n=10,380): RR=0.86 (95% CI, 0.78-0.96) Vertebral fractures (4 trials, n=42,185): RR=0.89 (95% CI, 0.74-1.09) Any fracture (10 trials, n=49,976); RR=0.95 (95% CI, 0.9-0.99)	Vitamin D alone had no effect on preventing hip fracture, non-vertebral fracture, vertebral fractures or any new fractures. Vitamin D with calcium supplements may prevent hip or any type of fracture.
Bolland *et al* (2014)	Selection criteria: *Inclusion:* RCTs included in meta-analyses studying vitamin D with the outcome fracture. *Exclusion:* cluster randomised trials, trials of hydroxylated vitamin D or vitamin D analogues, trials that included other interventions only in the vitamin D group, trials of fortified dairy products, and trials in populations with chronic comorbidity other than osteoporosis or frailty Outcome measure Fractures	22 RCTs (n=76,431) *Total fracture* Vitamin D only (15 RCTs): RR=0.97 (95% CI, 0.88-1.08) Vitamin D and calcium (10 RCTs): RR=0.92 (95% CI, 0.85-0.99) Vitamin D with and without calcium: RR=0.95 (95% CI, 0.88-1.02) p=0.13 *Hip fracture* Vitamin D only: RR=1.11 (95% CI, 0.97-1.27) Vitamin D and calcium: RR=0.84 (95% CI, 0.74-0.96) Vitamin D with and without calcium: RR=0.97 (95% CI. 0.86-1.08) p=0.55	Vitamin D with or without Ca had no effect on total fracture. Vitamin D had no effect on hip fracture but vitamin D + Ca reduced hip fractures. Trial sequential meta-analysis found that vitamin D supplementation (± Ca) does not reduce skeletal outcomes in community dwelling individuals by more than 15%.

Study	Methods	Results	Author's conclusions
Bischoff-Ferrari *et al* (2009)	Selection criteria *Inclusion:* Oral vitamin D supplementation (D2 or D3); minimum follow-up 1y; > 1 fracture; mean age ≥ 65 y; double-blinded; adherence reported; explanation of how fractures ascertained. *Exclusion*: Uncontrolled trials; observational studies, animal studies; studies with patients following organ transplantation or stroke, receiving steroid therapy or with Parkinson's disease or unstable health states. Outcome measure Nonvertebral or hip fracture.	12 double-blind RCTs (n=42,279) comparing oral vitamin D (± Ca) with calcium or placebo. Nonvertebral fractures (12 RCTs): RR = 0.86 (0.77-0.96) Evidence of heterogeneity (Q test: p=0.04); resolved after stratifying trials by received dose For 3 trials ≤ 10 µg, RR = 1.02 (0.92-1.15) (Q test, p=0.64) For 9 trials ≥ 10 µg, RR = 0.80 (0.72-0.89) (Q test, p=0.31) Hip fractures (8 RCTs): RR = 0.91 (0.78-1.05) Evidence of heterogeneity (Q test, p=0.08); resolved after stratifying trials by received dose For 3 trials ≤ 10 µg, RR = 1.09 (0.90-1.32) (Q test, p=0.81) For 5 trials ≥ 10 µg, RR = 0.82 (0.69-0.97) (Q test, p=0.18)	Non-vertebral fracture prevention with vitamin D is dose dependent. A higher received dose of supplemental vitamin D (12-19 µg daily) should reduce nonvertebral fractures by at least 20% and hip fractures by at least 18%.

Table 23: RCTs on vitamin D supplementation and increased fracture risk in adults ≥ 50y

Study/country	Population	Intervention & duration	Baseline 25(OH)D concentration (nmol/L)	12 m post intervention 25(OH)D concentration (nmol/L)	Results	Comments
Sanders *et al* (2010) Victoria, Australia	Women considered to be at high risk of fracture (n=2256) Age: 70+ y.	1. 12,500 µg D3 given as single annual dose orally. 2. Placebo Duration: 3-5 years	*(n=131)* Median (IQR): 49 (40-63)	≥ 74 1. 9.5% 2. 5.3% 51-74 1. 44.5% 2. 33.32% 26-50 1. 41.9% 2. 57.9% ≤ 25 1. 4% 2. 3.5%	Vitamin D vs placebo group: Incident rate ratio: 1.26 (95% CI, 1.00-1.59) (p=0.047)	Significant increase in fracture with high dose of vitamin D3.

Table 24: Prospective studies on association between 25(OH)D concentration and fracture risk in adults ≥ 50y

Study/Country	Population	Follow-up	Mean baseline 25(OH)D concentration (nmol/L)	Results	Comments
Cauley *et al* (2010) USA	Community-dwelling men; age: ≥ 65 y Case cohort study Study sub-cohort: 1,606 randomly selected men from the Osteoporotic Fractures in Men Study (MrOS). 435 incident nonspine fractures (n=112 in sub-cohort) observed in whole cohort (n=5,995). Non-spine fracture analysis n=1,929 (n=1,494 subcohort and n=435 fracture cases) Hip fracture analysis n=1,665 (n=1,584 subcohort and n=81 hip fracture cases).	5.3 y	No non-spine fracture sub-cohort 62.8 (19.6) Non-spine fracture patients 61.3 No hip fracture sub-cohort 62.8 (19.7) Hip fracture patients 52.8 (19.8)	*Relative Hazard of non-spine fracture by quartiles of 25(OH)D concentration (nmol/L) (Q1, 8-<47; Q2, 47-<63; Q3, 63-<70; Q4, ≥70)* Q1 vs Q4: 1.21 (95% CI, 0.86-1.65) Q2 vs Q4: 1.15 (95% CI, 0.84-1.56) Q3 vs Q4: 1.13 (95% CI, 0.81-1.53) (p for trend: 0.30) *Relative Hazard of hip fracture by quartiles of 25(OH)D concentration (nmol/L) Q1, 8-<47; Q2, 47-<63; Q3, 63-<70; Q4, ≥70)* Q1 vs Q4: 2.36 (95% CI 1.08-5.15) Q2 vs Q4: 1.48 (95% CI, 0.68-3.21) Q3 vs Q4: 0.98 (95% CI, 0.42-2.28) (p for trend: 0.O1)	No difference in 25(OH)D concentration in patients with non-spine fracture compared with the sub-cohort; total 25(OH)D, vitamin D3 and vitamin D2 unrelated to risk of non-spine fracture. Mean 25(OH)D concentration 16% lower in hip fracture patients compared with sub-cohort. One SD decrease in 25(OH)D associated with increased risk of hip fracture (multivariate HR = 1.60; 95% CI 1.18-2.17).
Cauley *et al* (2011) USA	Postmenopausal women (n=2,264; 1,132 cases; 1,132 controls) White (n=800) Black (n=762) Hispanic (n = 384) Asian (n=226) American Indian (n=92) Age: 50-79 y	8.6 y	White women *Cases:* 58.9 (23.7) *Controls:* 62.6 (24.2) Black women *Cases:* 44.4 (22.2) *Controls:* 42.9 (20.5) Hispanic women *Cases:* 52.4 (20.7) *Controls:* 53.7 (21.2) Asian women *Cases:* 62.6±23.7 *Controls:* 61.9±25.0 American Indian women *Cases:* 48.4±25.2 *Controls:* 51.7±25.7	*Odds ratio of fracture by 25(OH)D (nmol/L)* White women <50 vs 50-<75: 0.82 (95% CI, 0.58-1.16) < 50 vs ≥75: 0.56 (95% CI, 0.35-0.90) (ptrend: 0.008) Black women < 50 vs 50-<75: 1.48 (95% CI, 1.05-2.10) <50 vs ≥75: 1.33 (95% CI, 0.73-2.43) (ptrend 0.043) Hispanic women <50 vs 50-<75: 1.02 (95% CI, 0.69-1.79) <50 vs ≥75: 1.09 (95% CI, 0.50-3.37) (ptrend: 0.72) Asian women <50 vs 50-<75: 1.49 (95% CI, 0.76-2.50) < 50 vs ≥75: 1.66 (95% CI, 0.68-4.02) (ptrend: 0.22) American Indian women <50 vs 50-<75: 0.64 (95% CI, 0.15-2.79) <50 vs ≥75: 0.43 (95% CI, 0.09-2.08) (ptrend: 0.29)	*White women* 25(OH)D ≥75 nmol/L associated with a 44% significantly reduced risk of fracture. *Black women* 25(OH)D of 50 - < 75 nmol/L associated with a 48% significant increased risk of fracture. *Hispanic, Asian and American Indian women* No significant associations identified.

Study/Country	Population	Follow-up	Mean baseline 25(OH)D concentration (nmol/L)	Results	Comments
Nakamura *et al* (2011) Japan	Community-dwelling women (n=773) Mean age: 74.6y (4.4)	6 y	60 (± 17.6)	*Hazard ratios (HR) of limb or vertebral fractures by quartiles of 25(OH)D concentration* (nmol/L) (Q1, ≤ 47.7; Q2, ≥ 47.7-<59.2; Q3, ≥59.2-<71.0; Q4, ≥71.0) Q1 vs Q4: HR=2.82 (95% CI, 1.09-7.34) Q2 vs Q4: HR=1.84 (95% CI, 0.68-4.98) Q3 vs Q4: HR=2.82 (95% CI, 1.09-7.27) (p for trend: 0.1195) Pooled adjusted HR: 0.42 (95%CI, 0.18–0.99) comparing incidence in the fourth quartile (≥71.0 nmol/L) to the other three quartiles combined (<71.0 nmol/L).	Women in highest quartile of serum 25(OH)D had lower risk of fracture.
Rouzi *et al* (2012) Saudi Arabia	Healthy postmenopausal women (n=912) Mean age: 61.3 ± 7.2 y	5.2 y	34.27 ± 22.8	*Vertebral fracture risk* Serum 25(OH)D ≤17.9 vs > 45.1 nmol/L RR=1.63 (95% CI, 1.06-2.51; p <0.027)	Lowest quartile of serum 25(OH)D concentration independent risk factor for osteoporosis related fractures.
Barbour *et al* (2012)	Community dwelling white and black adults (n=2614). Age ≥70 y	6.4 y	Q1: <44.4 Q2: >44.4 – 60.8 Q3: >60.8 – 79.7 Q4: >79.7	Multivariable adjusted HRs (95% CIs): *Hip fracture:* Q1 vs Q4: HR=1.73 (0.80-3.75) Q2 vs Q4: HR=0.74 (0.31-1.79) Q3 vs Q4: HR=1.60 (0.83-3.10) p trend = 0.43 *Non-vertebral fractures* Q1 vs Q4: HR=1.36 (0.90-2.07) Q2 vs Q4: HR= (0.76-1.77) Q3 vs Q4: HR=1.23 (0.85-1.80) p trend = 0.43	No association between serum 25(OH)FD concentration and hip or non-vertebral fractures

Table 25: Meta-analyses of RCTs on effects of vitamin D supplementation on muscle strength and function in adults ≥ 50y

Study	Methods	Results	Conclusions
Muir *et al* (2011)	Selection criteria: *Inclusion*: RCTs of supplemental vitamin D or associated metabolite, +/- Ca, with a placebo or standard treatment arm; adults ≥ 60 y; physical performance measures of muscle strength, gait or balance measured at baseline end of study. *Exclusion*: Inclusion of an exercise intervention Outcome measure Muscle strength, gait and balance.	13 RCTs; mean age 78 ± 4.1 y; 7 studies in community dwelling adults, *Postural sway*: (3 RCTs: 2 RCTs vit D & Ca vs Ca, 1 RCT vit D only) Vit D supplement doses – 1 RCT 1,500 µg D2 single dose, 2 RCTs 20 µg/d D3. Standardised mean difference of -0.20 (95%CI -0.39,-0.01) p=0.04, I^2 = 0% *Timed up and go test* (3 RCTs vit D & Ca vs Ca) Vit D supplement doses (daily) – 1 RCT 10 µg D3, 1 RCT 20 µg D3, 1 RCT 25 µg D2. Standardised mean difference of -0.19 (95%CI -0.35,-0.02) p=0.03, I^2 = 0% *Knee extension strength* (3 RCTs: 2 RCTs vit D & Ca vs calcium, 1 RCT vit D only) Vit D supplement doses - 1 RCT 1,500µg D2 single dose, 1RCT 20 µg/d D3, 1 RCT 25 µg/d D2. Standardised mean difference = 0.05 (95%CI -0.11, 0.20) p=0.04, I^2 = 0%	Supplemental doses of 20-25 µg/d suggest beneficial effects on muscle strength and balance. An effect on gait was not demonstrated.
Stockton *et al* (2011)	Selection criteria *Inclusion:* RCTs in human adult participants. All forms and doses of vitamin D supplementation =/- Ca compared with placebo or standard care. *Exclusion: Not reported* Outcome measure Muscle strength.	17 RCTs; n=5072 *Grip strength* (7 RCTs – 5 RCTs vit D & Ca, 2 RCTs vit D only); vit D doses, 10-25µg D3 SMD = -0.02 (95%CI -0.15,0.11) 25OHD>25 nmol/L *Proximal trunk and upper limb strength* (1 RCT – vit D & Ca); vit D dose – 20 µg/d D3 Bench press: SMD = -0.23 (95% CI -0.66, 0.19) baseline 25(OH)D > 25 nmol/L Lateral pull downs: SMD = -0.32 (95% CI -0.75, 0.10) baseline 25(OH)D >25 nmol/L *Knee strength* (7 RCTs – 4 vit D & Ca, 1 vit D only); vit D supplement dose – 10-20 µg/d D3, 150,000 µg D3 (single dose), 7500 µg D2 (single dose) Knee extension strength: SMD = 0.10 (95% CI -0.02, 0.29) 25(OH)D > 25 nmol/L Knee flexion strength: SMD = 0.10 (95% CI -0.21, 0.41) *Leg press* (2 RCTs – vitamin D & Ca): vit D dose – 20-25 µg/d D3 SMD = 0.05 (95%CI -0.26, 0.39) *proximal lower limb strength* Overall: SMD = 0.1 (95%CI -0.01, 0.22) *Baseline 25(OH)D < 25 nmol/L* (2 RCTs): SMD = 3.53 (95% CI 2.18, 4.85)	Vitamin D did not have a significant effect on muscle strength in adults with baseline 25(OH)D >25 nmol/L. A limited number of studies demonstrated an increase in proximal muscle strength in adults with 25(OH)D < 25 nmol/L.
Beaudart *et al* (2014)	Selection criteria *Inclusion:* RCTs in English (1966-Jan 2014), control group comparable to treated group; muscle strength, mass or power measured before & after intervention in both groups. *Exclusion:* Reviews, non-randomised trials, animal studies, no placebo/control group, vit D used as part of complex nutritional supplementation regimen. Outcome measure Muscle function (muscle strength, mass & power).	30 RCTs, 5615 participants; mean age 61.1 years (10-99 years); 72% female Global muscle strength (29 RCTs): SMD 0.17 (95%CI 0.03, 0.31) (p=0.02) *Grip strength* (16 RCTs): SMD 0.01 (95%CI -0.06, 0.07) (p=0.87) *Lower limb* (16 RCTs): SMD 0.19 (95%CI 0.05, 0.34) (p=0.01) *Baseline 25(OH)D < 30 nmol/L*: SMD 0.47 (95% CI, -0.07 to 1.01) (p=0.02) Muscle mass (6 RCTs): SMD 0.058 (95%CI -0.118, 0.233) (p=0.520) Muscle power (5 studies: SMD 0.057 (95%CI -0.194, 0.308) (p=0.657)	Small significant positive effect of vitamin D on muscle strength. Improvement greater in particpants with 25(OH)D < 30 nmol/L. No significant effect on muscle mass or muscle power.

Table 26: RCTs (not included in above meta-analyses) on effect of vitamin D supplementation on muscle strength and function in adults ≥ 50y

Study/ Country	Study population	Intervention/study design	Mean baseline 25(OH)D (nmol/L)	Post intervention 25(OH)D (nmol/L)	Outcome	Results	Comments
Lips *et al* (2010) North America & Europe	Men & women (n=226) with serum 25(OH)D ≤50 and ≥ 15 nmol/l Mean age: 78y	1. 210 µg/wk D3 2. Placebo Duration: 16 weeks Double-blind	1. 34.2 ± 11.0 2. 35.2 ± 13.7	1. 65.4 2. Unchanged. Mean difference between groups of 32.4.	*Primary* - mediolateral body sway *Secondary* - short physical performance battery (SPPB)	<u>*Mediolateral sway*</u> – no change from baseline in either group. No significant treatment differences on basis of baseline 25(OH)D concentration (≤ 37 or > 37 nmol/L) <u>*SPPB*</u> – no significant difference in change in SPPB scores between intervention and placebo group	In the post hoc subgroup analysis, mediolateral sway was reduced in vitamin D supplemented patients with elevated sway at baseline (≥ 0.46 cm) (p=0.047).
Pirotta *et al* (2014) Australia	Men and women (n=26) with serum 25(OH)D concentration 25-60 nmol/L Mean age: Women – 66.1±4.0 y Men – 71.5±5.7 y	1. 50µg/d D3 2.Placebo Duration: 10 weeks Double blind	1. 46.4 ± 11.4 2. 48.5 ± 11.1	1. 81 2. No change	*Primary* – corticospinal excitability, intracortical inhibition *Secondary* – muscle strength (knee extension) & muscle power (stair climbing, four square step test, timed-up-and-go test)	Significant 8-11% increase in muscle strength in vitamin D group ($p < 0.05$) but changes not significantly different from the placebo group. No effect of vitamin D on muscle power.	Very small study.
Knutsen *et al* (2014) Norway	Adults (n=251) living in Oslo but born (or parents born) in Middle East, Africa, South Asia Age: 18-50 years	1. 10 µg/d D3 2. 25 µg/d D3 3. Placebo Duration: 16 weeks Double-blind.	1. 27 (15) 2. 27 (16) 3. 27 (15)	1.43 (17) 2.52 (20) 3.25 (12)	*Primary* - Jump height *Secondary* - chair rising test, handgrip strength.	Mean difference (95% CI) compared with placebo <u>Jump height</u> 1. -2.4 (-7.2, 2.4); p=0.24 2. -0.4 (-5.1, 4.4); p=0.85 <u>Handgrip strength</u> 1. -2.57 (-7.00, 1.86); p=0.25 2. 0.21 (-4.42, 4.85); p=0.93 <u>Chair test</u> 1. -0.53 (-4.09, 3.04); p=0.77 2. 0.02 (-3.97, 4.00); p=0.99	There was no significant improvement in tests of muscle strength in vitamin D supplemented groups compared to placebo group.

Table 27: Prospective studies on association between serum 25(OH) concentration and muscle strength and function in adults ≥ 50y

Reference/Country	Population Description	Follow-up	Muscle strength measure	Mean baseline 25(OH)D (nmol/L) (± SD)	Results
Bolland *et al* (2010) Australia	Community dwelling postmenopausal women (n=1471) Mean age: 74y	5y	Grip strength	50.5 ± 17.7 *(seasonally adjusted)*	No association between baseline seasonally adjusted 25(OH)D and grip strength.
Chan *et al* (2012) Hong Kong	Community dwelling men (n=714) Mean age: 72.8 ± 5.1y	4y	Grip strength, chair standing time, walking speed & appendicular skeletal muscle mass.	77.9 ± 20.5	No association between baseline 25(OH)D and change in appendicular skeletal muscle mass, grip strength, chair standing time or walking speed.
Houston *et al* (2011) USA	Community dwelling adults (n=988) (16.7% black) Mean age: 85.2 ± 3.6 y	3y	Short physical performance battery (SPPB): standing balance, repeated chair stand, gait speed scores; grip and knee extensor strength.	31% < 50 36% 50-74.6 33% ≥ 75	SPPB score significantly lower with 25(OH)D < 50 vs ≥ 75 nmol/L after adjustment for sociodemographic characteristics, season, health behaviours & chronic disease (p=0.006). Grip and knee extensor strength significantly lower in participants with 25(OH)D < 50 vs 75 nmol/L after adjustment for body weight, sociodemographic characteristics & season (p<0.01) and health behaviours and chronic conditions (p trend = 0.02).
Menant *et al* (2012) Australia	Community dwelling adults (n=463) Mean age: 78 ± 4.6 y	1y	Upper and lower limb strength, reaction time, postural sway, gait speed.	62.2 ± 24.6	After adjusting for age & BMI participants with 25(OH)D < 50 nmol/L showed weaker strength, slower reaction time, poorer leaning balance and slower gait speed.
Scott *et al* (2010) Australia	Community dwelling adults (n=686) Mean age: 62 ± 7 y	2.6y	Appendicular lean mass (ALM), leg strength and leg muscle quality (LMQ).	54.5 ± 19	% ALM, leg strength, LMQ) & physical activity was lower in participants with serum 25(OH)D < 50 nmol (p < 0.05 for all). After adjustment for confounders, baseline 25(OH)D concentration was significantly positively associated with change in leg strength (p=0.027) and leg muscle quality (p=0.003).
Michael *et al* (2011) US	Postmenopausal women (n=532) Age: 70.3 ±3.7	6y	Physical performance summary score derived from 3 tests: timed walk, chair-stand & grip strength.	48.2 ±21.4	Participants with serum 25(OH)D > 75 vs <25 nmol/L had significantly higher scores for physical performance (RR 2.64; 95% CI, 0.90-4.39; p trend < 0.001). Baseline 25(OH)D concentration did not reduce decline in physical performance over the 6 y follow-up period.

Reference/Country	Population Description	Follow-up	Muscle strength measure	Mean baseline 25(OH)D (nmol/L) (± SD)	Results
Houston *et al* (2012) USA	Community dwelling adults (n=2641). Mean age: 74.7 ± 2.9 y	4y	Short physical performance battery (SPPB): standing balance, chair-stands, timed walk). Modified physical performance battery (PPB): standing balance, leg stand, walk balance) Knee extensor strength Grip strength	1/3 < 50 2/3 < 75	Cross-sectional analysis: 25(OH)D < 50 vs ≥ 75 nmol/L associated with significantly poorer physical performance ($p<0.001$), slower gait speed ($p<0.0001$) and lower knee extensor and grip strength ($p<0.05$). Longitudinal analysis: participants with baseline 25(OH)D < 50 vs ≥75 nmol/L had poorer physical performance and slower gait speed at 2 and 4 y follow-up ($p<0.01$). Although physical performance and strength declined over 4 y ($p < 0.0001$), rate of decline was not associated with baseline 25(OH)D.

Table 28: Meta-analyses of RCTs on vitamin D supplementation (+/- calcium) on risk of falls in adults ≥ 50y

Study	Methods	Results	Author conclusions
Cameron *et al* (2012)	Selection criteria: *Inclusion:* All randomised trials of falls reduction in people ≥ 65y in nursing care facilities or hospitals, including quasi-randomised trials and those with inadequately concealed treatment allocation. *Exclusion:* Trials that reported only specific types of fall (e.g. injurious falls); trials that focused on intermediate outcomes (e.g. improved balance, strength). Outcome measure Rate of falls Risk of falling (no. of fallers)	**CARE FACILITIES** 6 RCTs (n=5186; mean age 84y): D3 (2), D2 (3) (2 included Ca); multivitamin that included D3 plus Ca (1). Dose: 5-27.5 µg/d D2, 20 µg/d D3 Baseline 25(OH)D (nmol/L) (mean): 22; 25-40 (57% of participants); 30 (median); 49; 59 (median, 47) (5-95th centile, 35-102); 36. *Rate of falls* 5 RCTs (n=4605); Rate Ratio (RaR) = 0.63 (0.46-0.86) (I2 = 72%) Vitamin D + calcium vs calcium (2 RCTs; n=747): RaR = 0.71 (0.56-0.90) (I^2 = 0%) Vitamin D2 vs usual care (no placebo) or placebo (2 RCTs; n=3765): RaR = 0.55 (0.19-1.64) (I^2 = 80%) Multivitamins (including D3 + Ca) vs placebo (1 RCT; n=91); RaR = 0.38 (0.20-0.71) *Risk of falling* (number of fallers) 6 RCTs; n = 5186); Risk Ratio (RR) = 0.99 (0.90-1.08) (I^2 = 12%) Vitamin D + calcium vs calcium (2 RCTs; n=747); RR = 0.85 (0.69-1.05) (I^2 = 0%) Vitamin D + calcium vs placebo (1 RCT; n=583); RR = 1.03 (0.90-1.18) Vitamin D2 vs usual care (no placebo) or placebo (2 RCTs; n=3765); RR = 0.80 (0.38-1.71) (I^2 = 58%) Multivitamins (including D3 + Ca) vs placebo 1 RCT (n=91); RaR = 0.82 (0.40-1.66) **HOSPITAL** *Risk of falling* Vitamin D + calcium vs calcium (1 RCT; n=203): RR=0.82 (0.59-1.14)	In care facilities, vitamin D supplementation is effective in reducing rate of falls *Authors state: Average serum vitamin D levels at baseline appeared to be low or very low in all 6 studies, therefore these results are only applicable to residents with low vitamin D levels.*

Study	Methods	Results	Author conclusions
Gillespie *et al* (2012)	Selection criteria: *Inclusion*: RCTs and quasi-randomised trials of interventions designed to reduce falls in people ≥60 y living in the community. Subgroup analysis of the effect of lower 25(OH)D concentration at baseline; 25(OH)D: (Dhesi 2004, ≤30 nmol/L; Pfeifer 2000, 25(OH)D ≤50 nmol/L; Pfeifer 2009, <78; Prince 2008, ≤59.9 nmol/L. *Exclusion*: Trials artificially inducing falls, e.g. during balance testing. Outcome measure Rate of falls Number of fallers (risk of falling).	14 RCTs (n=28,135): D2 (4); D3 (8); not specified (2); Ca co-supplementation(9); with vit D analogues (2). Baseline 25(OH)D (nmol/L) (mean): 74.7 (38.3); 31.8 (19.6); range 23.7-28; 72.6 (27.9); 79.3 (SD 24.7); 38.8 (15.6); 29.5 (range 6-85); 49.7; IG-37.4 (95% CI 34.9-44.9), CG-47.4 (95% CI 39.9-52.4); 25.2 (12.9); , 54.5 (18); 44.8 (12.7). **Vitamin D (+/- calcium) vs control/placebo/calcium** ***Rate of falls***: 7 RCTs (n=9324); Rate Ratio (RaR) = 1.0 (0.90-1.11) Vitamin D vs control or placebo (2 RCTs; n=2478): RaR = 1.14 (1.03-1.27) Vitamin D + Ca vs control or placebo (3 RCTs; n=6586): RaR = 0.96 (0.89-1.04) Vitamin D + Ca vs Ca 1 RCT; (n=137); RaR = 0.54 (0.30-0.98) ***Risk of falling (fallers)***: 13 RCTs (n=26,747): Risk Ratio (RR) = 0.96 (0.89-1.03) Vitamin D vs control or placebo (3 RCTs; n=4516): RaR = 1.08 (0.93-1.276) Vitamin D + Ca vs control or placebo (3 RCTs; n=6576): RaR = 0.98 (0.92-1.03) Vitamin D + Ca vs Ca (2 RCTs; n=379); RaR = 0.70 (0.53-0.28) **Participants with lower 25()OH)D concentrations** ***Rate of falls***: 2 RCTs(n=260) selecting on 25(OH)D ≤ 50 nmol/L: RaR = 0.57 (0.37-0.89) *(Dhesi, 2004 [24-28 nmol/L]; Pfeifer, 2000 [25 nmol/L])* 5 RCTs (n=9064) not selecting participants on 25(OH)D concentration: RaR = 1.02 (0.88-1.13) ***Risk of falling (fallers)*** 4 RCTs (n=562) selecting on 25(OH)D (≤ 78 nmol/L): RR = 0.70 (0.56-0.87) *(Dhesi, 2004 24-28 nmol/L; Pfeifer, 2000 [25 nmol/L]; Pfeifer, 2009 [55 nmol/L]; Prince, 2008 [45 nmol/L])* 9 RCTs (n=25,943) not selecting participants on 25(OH)D concentration: RR = 1.00 (0.93-1.07)	Overall, vitamin D supplementation does not appear to reduce falls but may be effective in people who have lower vitamin D levels before treatment.
Kalyani *et al* (2010)	Selection criteria: *Inclusion*: RCTs comparing vitamin D treatment with either Ca/ placebo/no treatment; mean age ≥ 60 y; no. participants with ≥ 1fall by treatment arm stated; explicit definition of fall; description of how falls ascertained. *Exclusion*: Studies of intramuscular vit D or including participants with neurological disabilities (e.g. Parkinson's disease or stroke). Outcome measure ≥ 1 fall during follow-up.	10 RCTs (n=2932; mean age 71-92y): D3 (6); D2 (3); Ca co-supplementation (7); with vitamin D analogue (1) Baseline 25(OH)D (nmol/L) (mean): IG-22.5/CG-25; 26; 30; 40 (median); 42/45/52; 45; 55; 66(f)/82(m); 75. ***Number of falls*** 10 RCTs (n=2932); Relative Risk (RR) = 0.86 (0.79–0.93) (I^2=7%, p=0.38*)* *Subgroup analysis by adjunctive calcium supplementation* Vitamin D alone (3 RCTs; n=856): RR = 0.94 (0.77–1.15) Vitamin D & calcium (7 RCTs; n=2076): RR = 0.83 (0.75–0.92) *Subgroup analyses by vitamin D dose* Dose < 20 µg/d (3 RCTs;n=950): RR = 1.01 (0.85-1.20) Dose ≥ 20 µg/d (7 RCTs; n=1679): RR = 0.80 (0.70-0.91) Significant reduction in no. of falls in following subgroups: community-dwelling (aged <80y); no history of fractures or falls; treatment duration > than 6 months.	Vitamin D treatment effectively reduces risk of falls in older adults. *(Authors state they were unable to investigate whether vitamin D treatment on fall prevention would also apply to populations that were not vitamin D deficient at baseline since 25(OH)D < 75 nmol/L in all included studies.)*

Study	Methods	Results	Author conclusions
Murad *et al* (2011)	Selection criteria: *Inclusion*: Randomised trials with adults who received vitamin D supplementation and comparison group that did not receive intervention. *Exclusion*: Studies using $1,25(OH)_2D$ or one of its analogues. Outcome measure Risk of at least 1 fall, i.e. fallers.	26 RCTs (n=45,782; mean age 76y; 78% female): D3 (12); D2 (9); D (5); Ca co-supplementation (15). Baseline 25(OH)D (nmol/L) (did not specify if mean or median): 20.7; 21.2 (13); 26; 31; 40; 42.2; 48.7; 52.4; 55; 55; < 60; 61.5 (30.3); 62.4-224.6; 70-75; 70-82.4; 71; 117.3. ***Risk of at least 1 fall (i.e. fallers)*** 26 RCTs: OR = 0.86 (0.77-0.96) (I^2= 66%) *Subgroup analysis by calcium co-administration* Vitamin D + calcium vs placebo (10 trials): OR = 0.83 (0.72– 0.93) Vitamin D vs. placebo (10 trials): OR = 0.97 (0.84 –1.11) Vitamin D + calcium vs calcium (10 trials): OR = 0.63 (0.50–0.81) *Subgroup analysis by vitamin D dose* > 20 μg/d (18 trials): OR = 0.82 (0.73– 0.93) < 20 μg/d (8 trials): OR = 1.00 (0.72–1.37) *Subgroup analysis by baseline vitamin D deficiency status (deficiency not specified)* Not deficient (20 trials): OR = 0.90 (0.81– 0.99) Deficient (6 trials): OR = 0.53 (0.39–0.72) ***Number of falls*** OR = 0.79 (0.70-0.88) (I^2= 90%)	Vitamin D combined with calcium reduces risk of falls. Fall reduction in studies without calcium coadministration did not reach statistical significance.
Bolland *et al* (2014)	Selection criteria: *Inclusion*: randomised trials of vitamin D supplementation with outcome data for falls published since Jan 2009. *Exclusion:* cluster randomised trials, trials using hydroxylated vitamin D or vitamin D analogues, other interventions included only in vitamin D gp, & trials in populations with chronic comorbidity other than osteoporosis or frailty. Outcome measure Falls	20 RCTs (n=29,535; mean age range 74-89y) Baseline 25(OH)D (nmol/L) (did not specify if mean or median) nit D gp/no vit D gp: 26/25; 22/23; 31/29, 38/48, 27/25, 29/30, 38/60, 75/72, 48/53, 25/22, 57/43, 45/44, 55/54, 50/49, 53/45, 65/67. ***Risk of falls*** Vitamin D with/without calcium (20 RCTs): RR = 0.96 (0.91-1.01; p=0.12) (I^2 = 55) Vitamin D (16 RCTs): RR = 0.95 (0.89-1.02) (I^2 = 61%) Vitamin D and calcium (6 RCTs): RR = 0.95 (0.89-1.03) (I^2 = 38%) *Subgroup analysis by baseline 25(OH)D concentration* Vitamin D with/without calcium: 25(OH)D < 50 nmol/L (12 RCTs): RR = 0.97 (0.89-1.05); 25(OH)D ≥ 50 nmol/L (4 RCTs): RR = 0.91 (0.77-1.06)	No effect of vitamin D supplementation, with or without calcium, on risk of falls.

Table 29: RCTS on the effect of vitamin D supplementation on risk of falls in adults ≥ 50y

Reference/Country	Population	Intervention/Duration	Baseline 25(OH)D concentration (nmol/L)	Results	Authors Conclusions
Bischoff-Ferrari *et al* (2016) Switzerland	Community-dwelling men and women (n=200) with a low-trauma fall in previous 12 months. Age: ≥ 70 y; mean age: 78 y	1. 600 µg vitamin D3/month 2. 1,500 µg vitamin D3/month 3. 600 µg vitamin D3 plus 300 µg 25(OH)D/month Duration: 12 months	1. 46.7 ± 24.5 2. 52.2 ± 23.0 3. 45.9 ± 19.0	Lower extremity function No difference in adjusted mean SPPB function decline score changes between treatment groups at 12 months (p=0.26) Incidence of falls: Significantly increased incidence in group 2 (66.9%; 95% CI, 54.4% - 77.5%) and group 3 (66.1%; CI, 53.5% - 76.8%) compared with group 1 (control) (47.9%; 95% CI, 35.8% - 60.3%) (p=0.048)	Higher monthly doses of vitamin D had no benefit on lower extremity function and were associated with increased risk of falls compared with the control group.

Table 30: Cohort studies on the association between serum 25(OH)D concentration and risk of falls in adults ≥ 50y

Reference/Country	Population Description	Follow-up	Mean baseline 25(OH)D (nmol/L) (SD)	Results	Comments
Menant *et al* (2012)	Community dwelling adults (n=463) Mean age: 78 ± 4.6 y	1y	62.2 ± 24.6	No difference in 25(OH)D between faller and non-faller groups (60.7 ±24.3 vs 63.2 ± 24.9 nmol/L; p=0.27) Serum 25(OH)D concentration < 50 nmol/L: Men: IRR=1.93 (95% CI, 1.19-3.15; p=0.008) Women: IRR=0.83 (95% CI, 0.56-1.23; p=0.362)	25(OH)D < 50 nmol/L was a significant risk factor for falls among men but not among women.

NON-MUSCULOSKELETAL HEALTH OUTCOMES

Pregnancy and lactation: non skeletal outcomes in mother and baby

Table 31: Systematic reviews on maternal vitamin D supplementation/25(OH)D concentration on maternal and offspring outcomes

Study	Methods	Results	Authors Conclusions
De-Regil *et al* (2016)	Selection criteria: *Inclusion:* RCTs; pregnant women of any gestational or chronological age; interventions involving vitamin D supplementation during pregnancy irrespective of dose, duration or time of commencement of supplementation. *Exclusion:* pregnant women with pre-existing conditions (e.g. gestational diabetes) Outcomes *Maternal:* pre-eclampsia, gestational diabetes, 25(OH)D concentration at term, adverse effects (primary); impaired glucose tolerance, caesarean section, gestational hypertension, maternal death (secondary) *Infant*: pre-term birth (< 37 wks gestation); low birth weight (<2500 g) (primary); birth length, head circumference, birth weight, admission to special care, still birth, neonatal death, apgar score < 7 at 5 minutes, neonatal infection, very pre-term birth (< 32 weeks' gestation).	9 intervention trials compared effect of vitamin D alone vs no supplementation or a placebo; 6 compared effects of vitamin D and calcium with no supplementation. ***Vitamin D alone versus no supplementation or a placebo*** Maternal vitamin D concentration at term (25(OH)D) (7 trials, n=868): 47.24 nmol/L higher in intervention v control group; low grade quality of evidence, high heterogeneity between studies Pre-eclampsia (2 trials, n=219): RR=0.52 (95% CI, 0.25-1.05); low quality of evidence Gestational diabetes (2 trials, n=219): RR=0.43 (95% CI, 0.05-3.45); very low quality of evidence Pre-term birth (3 trials, n=477): RR=0.36 (95% CI, 0.14-0.93); moderate quality of evidence Low birth weight (3 trials, n=493); RR=0.40 (95% CI, 0.24-0.67); moderate quality of evidence Caesarian section (2 trials, n=312); RR=0.95 (95% CI, 0.69-1.31) Stillbirths (3 trials, n=540); RR=0.35 (95% CI, 0.06, 1.99) Neonatal deaths (2 trials, n=282); RR=0.27 (95% CI, 0.04-1.67) Infant length (4 trials, n=638); MD: 0.70 (95% CI, 0.02-1.43) Head circumference at birth (4 trials, n=638): MD: 0.43 (95% CI, 0.03-0.83) ***Vitamin D and calcium versus no supplementation or a placebo*** Pre-eclampsia (3 trials, n=1,114): RR=0.51 (95% CI, 0.32-0.80); moderate quality of evidence Pre-term birth (3 trials, n=798): RR=1.57 (95% CI, 1.02-2.43) Maternal vitamin D concentration at term, gestational diabetes, adverse effects and low birth weight were not reported in any trial or were reported only by one study.	Supplementing pregnant women with vitamin D increased serum 25(OH)D at term and may reduce risk of pre-eclampsia, low birth weight and pre-term birth. However, when vitamin D and calcium are combined, the risk of pre-term birth is increased. Risk of bias in the majority of trials was unclear and many studies were at high risk of bias for blinding and attrition rates. Results should be interpreted with caution.

Study	Methods	Results	Authors Conclusions
Harvey *et al* (2014)	Selection criteria: *Inclusion:* RCTs, cohort studies, case control studies of pregnant women or pregnant women and their offspring; included measure of 25(OH)D concentration or supplementation with vitamin D or vitamin D containing food. *Exclusion:* ecological and animal studies, not written in English, did not measure maternal 25(OH)D concentration in or immediately after pregnancy or did not supplement participants with vitamin D in pregnancy, or where an outcome of interest was not assessed. Outcomes *Primary:* Neonatal hypocalcaemia, rickets in the offspring and offspring bone mass; maternal osteomalacia *Secondary:* Offspring body composition (incl. birth weight, birth length, head circumference, anthropometry, small for gestational age, preterm birth and later offspring outcomes (incl asthma & atopy, blood pressure & type 1 diabetes); maternal quality of life (incl. pre-eclampsia, gestational diabetes, risk of caesarean section & bacterial vaginosis).	Birth weight (BW) 9 intervention trials: 3 reported significantly greater BW in offspring of supplemented mothers 19 observational studies (14 cohort; 5 x-sectional); 6 studies reported significant +ve relationship between maternal 25(OH)D & offspring BW, 1 reported a significant negative association. Meta-analysis of 3 observational studies found weak positive associations between maternal 25(OH)D & offspring BW: Change in BW per 10% increase in vitamin D: 5.63 (95% CI, 1.11-10.16) Birth length (BL) 2 intervention trials: 1 reported offspring BL of vit D supplemented women was greater than that for unsupplemented women; the other reported no association. 12 observational studies (9 cohort, 3 x-sectional): 2 reported significant +ve association between maternal vitamin D status and offspring BL but neither measured maternal 25(OH)D during pregnancy; 10 studies found no relationship. Head circumference (HC) 2 intervention studies: 1 reported significantly greater offspring HC in supplemented mothers; 1 reported no effect of supplementation. 11 observational studies (8 cohort, 3 cross-sectional) – none found association between maternal 25(OH)D & offspring HC. Small for gestational age (SGA) 2 intervention studies: both reported no significant difference in SGA risk between vit D supplemented and unsupplemented mothers. 7 observational studies: 1 reported significant risk of SGA if maternal 25(OH)D <30 nmol/L; 1 reported U-shaped relationship in white women only with lowest risk with maternal 25(OH)D between 60-80 nmol/L; 3rd study of women with pre-eclampsia found lower 25(OH)D in those women with SGA infants compared to control group; 4 studies found no association. Preeclampsia 11 observational studies (4 cohorts, 6 case-control, 1 x-sectional study): 5 found significant inverse association between maternal 25(OH)D & preeclampsia risk; 6 found no relationship. Meta-analysis of 4 studies: OR 0.78 (95% CI, 0.59-1.05). Maternal gestational diabetes 8 observational studies (3 cohorts, 4 case-control, 1 x-sectional study): 3 found inverse relationship between maternal 25(OH)D and risk of gestational diabetes.	Some evidence from observational studies to support a positive relationship between maternal 25(OH)D and offspring BW. In no single disease area did the evidence base unequivocally support use of vitamin D supplementation during pregnancy.

Table 32: Intervention studies on effect of vitamin D supplementation during pregnancy on maternal non-skeletal reproductive health outcomes

Study/Country	Population/Design of study	Design	Intervention/Duration	Mean 25(0H)D maternal baseline (nmol/L)	Mean 25(OH)D cord (nmol/L)	Results/Comments
Hollis *et al* (2011) S Carolina US	Pregnant women 12-16 weeks gestation (n=350)	RCT	1. 10µg/d 2. 50µg/d 3. 100µg/d From 12-16 wks until delivery	79 (±86) 98 (±34) 111 (±40)	45 (±25) 57 (±25) 66 (±26)	No significant effect of vitamin D on risk of instrumental delivery; however, no control group.

Table 33: Intervention studies on effect of vitamin D supplementation during pregnancy on neonatal hypocalcaemia

Study/Country	Population/Design of study	Design	Intervention/Duration	Mean 25(0H)D maternal baseline (nmol/L)	Mean 25(OH)D cord (nmol/L)	Results/Comments
Cockburn *et al* (1980) Edinburgh Scotland	Pregnant women (n=1139)	Non-RCT	1. 10µg/d D2 2. Placebo From 12 wks of pregnancy to delivery	24 weeks 1. 39 2. 32.5 34 weeks 1. 44.5 2. 38.5 Delivery 1. 42.8 2. 32.5	1. 28 2. 20	Neonatal hypocalcaemia (defined as plasma Ca^{2+} < 1.85 mmol/L) occurred in 6% of the intervention group infants and 13% of controls ($p < 0.005$).
Brooke *et al* (1981) London UK	South Asian women in their last trimester (n=126)	RCT	1. 25 µg/day 2. Placebo Throughout last trimester of pregnancy	1. 168 2. 16	1. 138 (SE=10.8) 2. 10 (SE=2.0)	5 infants in control gp but 0 n treatment gp developed hypocalcaemia (plasma Ca2+ < 1.8 nmol/L). 25(OH)D concentrations in cord & maternal plasma greatly exceed those observed in other studies.
Delvin *et al* (1986) Lyon France	Pregnant women at the end of 1st trimester (n=40)	RCT	1. 25 µg/d D3 2. No treatment Throughout last trimester.	1. 45 (5) 2. 17.5 (2.5)	1. 32.5 (2.5) 2. 12.5 (2.5) (serum concentration 4 days after birth)	Significant difference ($p<0.002$) in serum Ca at 4d of age in both grps although to lesser extent in infants of supplemented mothers ($p<0.05$).

Table 34: Intervention studies on effect of vitamin D supplementation during pregnancy on birth weight, birth length, small for gestational age (SGA)

Study/Country	Population	Design	Intervention/Duration	Mean 25(0H)D maternal baseline (nmol/L)	Mean 25(OH)D cord (nmol/L)	Results/Comments
Wagner *et al* 2013a US	Pregnant women 12-16 weeks gestation (n=257)	RCT	1. 50µg/d 2. 100µg/d From 12-16 wks until delivery.	Baseline: 56.7 (24.2) Before delivery: 1. 90.4 (37.4) 2. 94.6 (33.7) (no significant difference between groups)	1. 55.2 (25.7) 2. 67.4 (33.2) (p=0.02)	No difference between 2 groups in birth weight, gestation or neonatal health.
Cooper *et al* (2016) Southhampton, Oxford & Sheffield UK	Pregnant women with serum 25(OH)D of 25-100 nmol/L & serum calcium <2.75 mmol/L at 10-17 weeks gestation (n=1134) & their infants Mean age: 30.5 y (5.2)	RCT	Randomly assigned at 14 weeks gestation to: 1. 25 µg/d 2. placebo (All able to continue self-administration of antenatal vitamins containing ≤ 10µg /d vitamin D) Duration: until delivery	<u>Baseline</u> 1. 46.7 (17.7) 2. 45.9 (17.0) (difference: p>0.05) <u>34 weeks gestation</u> 1. 67.8 (22.1) 2. 43.3 (22.3) (difference: p<0.0001)	Not reported	No difference in birth weight, birth length and head circumference between groups.

Table 35: Cohort studies on associations between 25(OH)D concentration and birth weight, birth length, SGA

Study/country	Population description	Follow up	Mean 25(OH)D (nmol/L)	Results	Comments
Burris *et al* (2012) USA	White (n=1067) & black (n=236) mother infant pairs	2nd trimester to delivery.	Mothers (2nd trimester) Black: 46 (22)/White: 62 (20) Infants (cord): Black: 31 (16)/White: 51 (18)	Odds of SGA maternal 25(OH)D <25 vs ≥25 nmol/L: OR 3.17 (95%CI, 1.16-8.63). infant cord 25(OH)D <25 vs ≥25 nmol/L: OR 4.64 (95%CI, 1.61-13.36)	2nd trimester 25(OH)D <25 vs ≥25 nmol/L associated with higher odds of SGA in black and white infants.

Table 36: Observational studies on association between maternal 25(OH)D concentration and later growth and development of the offspring

Study/country	Population description	Follow up	Mean 25(OH)D (nmol/L)	Results	Comments
Cognitive and psychological development					
Gale *et al* (2008) UK	Pregnant women at 17 wks gestation (n=466)	Offspring followed up to age 9 y	Median at 32.6 wks gestation (IQR, 32-33.4 wks): 50 (IQR, 30-75.3)	No difference in IQ at 9y according to maternal 25(OH)D concentration Psychological health at age 9y 30-50 nmol/L: OR 2.11 (95%CI 0.59, 7.62) <30 vs 51-75 nmol/L: OR 2.44 (95%CI 0.69, 8.64) <30 vs >75 nmol/L: OR 0.75 (95%CI 0.16, 3.58)	No association between maternal 25(OH)D and measures of cognitive function or psychological health.
Whitehouse *et al (*2012) Western Australia	White pregnant women at 18 wks gestation (n=743)	Offspring followed up to age 17 y	Q1 = 36.8 (±7.1) Q2 = 53.1 (±3.9) Q3 = 65.1(±3.4) Q4 = 83.5nmol/L (±12.1)	Association between maternal 25(OH)D & offspring language impairment (at 5 & 10y) OR (95% CI) (Q4 Ref) Q3 OR 1.44 (0.74, 2.8; p=0.28) Q2 OR 1.35 (0.71, 2.57; p=0.36) Q1 OR 1.97 (91.00, 3.93; p<0.05)	Significant increased risk of women with 25(OH)D <46 vs 75 nmol/L during pregnancy having a child with clinically significant language difficulties.
Later growth					
Brooke *et al* (1981)	South Asian women (n=126) supplemented with vitamin D2 (25µg/d) or placebo in last trimester.	Offspring followed for 1y	At term: 1. 168 (±12.5) 2. 16.2 (±2.7)	Head circumference (cm) at 12 months: 1. 45.9 (±1.6) 2. 45.7 (±1.4)	At age 1y, no significant difference in offspring head circumference between groups.
Gale *et al* (2008) Southampton UK	Pregnant women at 17 wks gestation (n=466)	Offspring followed up to age 9 y	Median at 32.6 wks gestation (IQR, 32-33.4 wks): 50 (IQR, 30-75.3)	Head circumference at age 9y (cm) according to maternal 25(OH)D (nmol/L) <30: 52.6 30-50: 53.2 51-75: 53.4 >75 nmol/L 53.6 p=0.012	Head circumference significantly greater at age 9 y in offspring of mothers with 25(OH)D >75 vs < 30 nmol/L in 3rd trimester.
Respiratory disease					
Gale *et al* (2008) Southampton UK	Pregnant women at 17 wks gestation (n=466)	Offspring followed up to age 9 y	Median at 32.6 wks gestation (IQR, 32-33.4 wks): 50 (IQR, 30-75.3)	Asthma risk at 9 y according to maternal 25(OH)D <30 vs >75 nmol/L: OR, 5.40 (95% CI, 1.09-26.6; p=0.038)	Children whose mother had 25(OH)D > 75 compared to < 30 nmol/L during pregnancy had an increased risk of asthma at 9y.

Table 37: RCTs of vitamin D supplementation and cancer risk

Study/country	Study population	Intervention	Mean baseline **25(OH)D** (nmol/L)	Mean post intervention **25(OH)D** (nmol/L)	Cancer outcome	Results **Cases - Intervention/placebo**	Comments
Trivedi *et al* (2003) Suffolk, UK	Men & women (n=2686) Recruited from British doctors register & general practice register. Age: 65-85y	1. 2500 µg/4 months (equivalent 21 µg/d) 2. Placebo Duration: 5 y	Not reported	Not reported	Colon cancer mortality	1. 7 cases 2. 11 cases HR: 0.62 (95% CI 0.24-1.60) p=0.33	No effect on cancer incidence.
					All cancer mortality	1. 63 cases 2. 72 cases HR: 0.86 (95%CI, 0.61-1.20)	No effect on cancer incidence
Lappe *et al* (2007) Nebraska, USA	Postmenopausal women (n= 734) Age: >55 y	1. 27.5 µg/d D3 + 1450 mg/d Ca 2. 1400-1500mg/d Ca 3. Placebo Duration: 4 y	1. 71.8 (±20) 2. 71.6 (±20.5) 3. 72.1 (±20.7)	At 12 months: 1. 96 (±21.4) 2. 71 (±20.3) 3. 71.1 (±19.8)	All cancers	1. 13 cases 2. 17 cases 3. 20 cases RR = 0.40 (95% CI, 0.20-0.82)	Reduced risk of cancer in the vitamin D and calcium group and calcium only group.
Wactawski-Wende *et al* (2006) USA	Postmenopausal women (n=36,282) Age: 50-79y	1. 10 µg D3 with 1000mg/d Ca 2. Placebo Duration: 7 years	Not reported	Not reported	Colorectal	1. 168 cases 2. 154 cases HR = 1.08 (95% CI, 0.86-1.34) p=0.51	No effect on cancer incidence.
Avenell *et al* (2012) UK	Participants recruited from fracture clinics (n=5292) Age: >70 years	1. 20 µg D3/d 2. 1000 mg/d Ca 3. 20 µg D3 + 1000 mg/d Ca 4. Placebo Duration: 24-62 m intervention & 3 years post intervention	Not reported	Not reported	All cancers	2 338 cases (with vitamin D or calcium) 315 cases (without vitamin D or calcium) HR = 1.07 (95% CI, 0.92-1.25) p=0.376	No effect on cancer incidence.

Table 38: Meta analyses of RCTs and observational studies on vitamin D supplementation/25(OH)D concentration and cancer risk

Study	Methods	Results	Conclusions
Total cancer			
Keum & Giovannucci (2014)	Selection criteria: *Inclusion*: RCTs on effect of vitamin D supplementation (± calcium) on total cancer incidence or mortality. *Exclusion:* abstracts and unpublished results. Outcome measure: Total cancer incidence or mortality	Total cancer incidence: 4 RCTs (n=45,151; 4333 cases) RR = 1.00 (95% CI 0.94-1.06; p=0.998) Total cancer mortality: 3 RCTs (n=44,260; 1190 deaths) RR = 0.88 (95% CI, 0.78- 0.98; p=0.02)	Vitamin D supplementation had no significant effect on total cancer incidence but significantly reduced total cancer mortality.
Colorectal cancer			
Gandini *et al (*2011)	Selection criteria: *Inclusion*: Case-control & cohort studies reporting relative risks or crude data for serum 25(OH)D concentrations. *Exclusion*: Studies using predictive models or serum $1,25(OH)_2D$ concentrations. Ecological studies, case reports, reviews and editorial. Outcome measure: Colorectal cancer	9 studies, 2630 cases Per 25 nmol/L increase in 25(OH)D concentration: RR =0.85 (95%CI 0.79, 0.92)	Significant inverse relationship between 25(OH)D and colorectal cancer risk.
Chung et al (2011)	Selection criteria: *Inclusion:* RCTs comparing vit D supplementation with/without calcium against no supplementation or placebo. Prospective cohort or nested case-control studies examining association between 25(OH)D with cancer outcomes. *Exclusion:* pregnant women; RCTs comparing different vit D supplementation dosages without control group. Short term RCTs & trials (< 1 month) using synthetic vitamin D analogues. Outcome measure: Cancer incidence and mortality.	1 trial; n=36,282; colorectal cancer incidence: Incidence: HR=1.08 (95% CI, 0.86-1.34); Mortality: HR=0.82 (95% CI, 0.52-1.29) 9 cohort studies, 3,136 cases Per 10nmol/L increase in 25(OH)D: OR=0.94 (95% CI, 0.91-0.97)	
Breast cancer			
Sperati *et al* 2013	Selection criteria: *Inclusion:* RCTs which administered single agent compared with placebo/no treatment or as part of combined regimens incl. lifestyle modifications if co-intervent on same in all groups. For multi-arm RCTs, all pairwise comparisons with arms differing by vitamin D use only were included. *Exclusion:* RCTs with pregnant or lactating women. Outcome measure: Breast cancer incidence & mortality.	2 trials (n=5372) RR = 1.11 (95% CI, 0.74-1.68).	No effect of vitamin D supplementation on risk of breast cancer

Study	Methods	Results	Conclusions
Kim & Je (2014)	Selection criteria: *Inclusion:* original data from cohort/nested case-control studies of vitamin D intake or blood 25(OH)D concentrations; provided relative risks & confidence intervals. *Exclusion:* not reported. Outcome measure: Breast cancer incidence or mortality	*Breast cancer risk* (13 nested case control/1 cohort study; 9526 cases) 25(OH)D concentration (nmol/L): Highest (>77.4) vs lowest (<44.9): RR= 0.92 (95% CI, 0.83-1.02) (I^2=27.3%; p=0.16) Per 25 nmol/L increment: RR= 0.98 (95% CI, 0.96-1.00) *Breast cancer mortality* – 6 prospective studies (n=4,556; 301 deaths) 25(OH)D concentration (nmol/L): Highest (>72.6) vs lowest (<52.4) RR = 0.58 (95% CI, 0.40-0.85) Per 25 nmol/L increment: RR = 0.88 (95% CI, 0.79-0.98)	Among breast cancer patients, 25(OH)D > 72.6 vs < 52.4 nmol/L was significantly associated with better breast cancer survival.
Prostate cancer			
Gilbert *et al* (2011)	Selection criteria: *Inclusion:* primary epidemiological data & either: serum 25(OH)D or 1,25$(OH)_2$D) concentrations or dietary and/or supplement vitamin D intake. 25(OH)D measured prior to cancer diagnosis. *Exclusion:* Animal or case studies. Chemotherapy also included. Studies on vit D & prostate cancer survival. Outcome measure: Prostate cancer	*Total prostate cancer risk (*14 cohort/nested case control studies; 4353 cases) per 25 nmol/L increase in 25(OH)D OR = 1.04 (95% CI, 0.99-1.10; p=0.12) *Aggressive prostate cancer risk* (6 studies; 871 cases) per 25 nmol/L increase in 25(OH)D OR = 0.98 (95% CI, 0.84-1.15; p=0.78)	No association between 25(OH)D and total and aggressive prostate cancer risk.
Ovarian cancer			
Yin *et al* (2011)	Selection criteria: *Inclusion:* Original longitudinal human studies. *Exclusion:* Reviews, ecological & case control studies; only vitamin D intake reported; only ovarian cancer mortality assessed. Outcome measure Ovarian cancer incidence & mortality.	10 longitudinal studies: (n=2488; 883 ovarian cancer cases). Ovarian cancer incidence, per 50 nmol/L increase in 25(OH)D: RR 0.83 (95%CI 0.63, 1.08) p=0.16	No significant association between 25(OH)D and ovarian cancer risk.
Non melanoma skin cancer			
Caini *et al* (2014)	Selection criteria: *Inclusion: RCTs, c*ase-control, nested case control, cohort studies examining association between vitamin D intake/ 25(OH)D concentration & cutaneous melanoma/non melanoma skin cancer risk which included measure of relative risk with 95% CI or another measure of statistical uncertainty & measure of tumour thickness. *Exclusion:* Ecological studies, case reports, reviews, editorials. Outcome measure Cutaneous melanoma & non melanoma skin cancer.	Relative risk (95% CI) highest versus lowest quantile of 25(OH)D *Cutaneous melanoma:* (3 cohort/1 case-control studies; 392 cases) RR = 1.46 (0.60-3.53) (I^2 = 54%) *Basal cell carcinoma*: (4 cohort studies; 1221 cases) RR = 1.82 (1.38-2.40) (I^2 = 0%) *Squamous cell carcinoma*: (3 cohort studies; 328 cases) RR = 1.68 (95% CI 0.44-6.39) (I^2 = 81%) *Non melanoma skin cancer*: (2 cohort studies; 768 cases): RR = 1.64 (95% CI 1.02-2.65) (I^2 =81%)	No association between 25(OH)D & cutaneous melanoma risk. Higher 25(OH)D concentration associated with significant increase in basal cell skin cancer & non melanoma skin cancer risk.

Table 39: Prospective studies on 25(OH)D concentration and cancer risk

Study/Country	Population	Mean follow up	Mean 25(OH)D (nmol/L)	Results/comments
Colorectal				
Woolcott *et al* (2010) USA	Adults from multi-ethnic cohort (of Japanese, Latino, African American, White, and Native Hawaiian ancestry) (n= 229 cases & 434 controls) Mean age: 69 y	1.7 years	Cases: 57.9 ±25.2 Controls: 62.4 ± 24.7	Association between 25(OH)D (nmol/L) and colorectal cancer risk <41.9 vs 41.9 to <55.4: OR = 0.63 (95% CI 0.37, 1.08) <41.9 vs 55.4 to <67.1: OR = 0.54 (95% CI 0.32, 0.93) <41.9 vs 67.1 to < 81.9: OR = 0.62 (95% CI 0.36, 1.07) <41.9 vs ≥ 81.9 = OR 0.60 (95% CI 0.33, 1.07) per doubling of 25(OH)D: OR = 0.68 (95% CI 0.51, 0.92; p=0.01) Inverse association between 25(OH)D concentration and colorectal cancer risk.
Weinstein *et al* (2011) Finland	Men taking part in the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention study (n=428 cases & 428 controls) Age range: 50-69 y	6.1 years (median)	*Median:* Colon cancer Cases 32.4 (22.5-49.3) Controls 29.6 (20.5-45.7) Rectum cancer Cases 34.6 (21.3-50.9) Controls 31.2 (22.1-45.6)	OR (95% CI) for highest 25(OH)D category(>75 nmol/L) vs lowest (< 25 nmol/L) Colorectal cancer: 1.47 (0.72-3.01) Colon cancer: 2.28 (0.77-6.78) Rectal cancer: 1.19 (0.43-3.28) No significant association between serum 25(OH)D & colorectal cancer risk.
Lee *et al* (2011) USA	Men taking part in Physician's Health study (n= 229 cases & 389 controls) Age range: 40-84 y	8.9 years (median)	*Median* Cases 66.4 Controls 63.9	Colorectal cancer: Highest 25(OH)D vs lowest quantile (measurement not provided): OR: 1.08 (95% CI 0.62-1.87) Colon cancer: Highest 25(OH)D quantile (median 91.9 nmol/L) vs lowest (median < 37.2 nmol/L): OR: 1.38 (95% CI, 0.73-2.64) Rectal cancer: Highest 25(OH)D quantile (median, 97.8 nmol/L) vs lowest (median, 44.7 nmol/L): OR: 0.45 (95% CI, 0.14-1.46) No significant associations.
Neuhouser *et al* (2012) USA	Postmenopausal women (n= 310 cases & 310 controls) Age range: 50-79 y	7-12 y	Not provided.	Highest serum 25(OH)D quartile (≥64.5 nmol/L) vs lowest quartile (< 32.7 nmol/L) OR = 4.45 (95% CI, 1.96-10.10) (p=0.003).
Weinstein *et al* (2014)	Men in Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (n=476 cases & 476 controls) Age 55-74 y	5.6 years (0.3-11.4) (Median & interdecile range)	*Median (IQR)* Cases: 52.3 (39.6-64.8) Controls 56.5 (41.2-71.1)	Serum 25(OH)D > 75 vs <25 nmol/L: OR = 0.87 (0.58-1.33) Per 25 nmol/L increase in 25(OH)D concentration: OR = 0.77 (95% CI, 0.65-0.91)
Breast				
Kim *et al* 2014 USA	Postmenopausal women in the multi-ethnic cohort (White, African-American, Native Hawaiian, Japanese & Latino) (n=707 cases & 07 controls). Age: 45–75 y	3.1 y	White: Cases, 87.1 (25.5)/controls, 93.4 (24.2) African American Cases, 64.1 (30.2)/controls, 65.9 (28.2) Native Hawaiian Cases, 81.4 (21.7)/controls, 83.4 (30.5) Japanese Cases, 81.6 (23.2)/controls, 81.4 (25.5) Latino Cases, 69.1 (23.5)/controls, 67.6 (23.5)	*Per 25nmol/L increase* White: OR=0.66 (95% CI, 0.48-0.90) African-American: OR=1.08 (95% CI, 0.79-1.47) Native Hawaiian: OR=0.79 (95% CI, 0.52-1.20) Japanese: OR=1.04 (95% CI, 0.84-1.28) Latino: OR=1.17 (95% CI, 0.84-1.64) Overall, p=0.086; whites vs others, p=0.051 25 nmol/L increase in 25(OH)D associated with reduced breast cancer risk in whites.

Study/Country	Population	Mean follow up	Mean 25(OH)D (nmol/L)	Results/comments
Prostate				
Meyer *et al* (2013) Norway	Males (n=2106 cases & 2106 controls) Mean age: 48.2 ± 9.2y	16.1 y (median)	Cases: 64.4 ± 22.2 Controls: 62.4 ± 22.3	Relative risks (95% CI) by 25(OH)D concentration (nmol/L) <30 0.82 (0.58, 1.15) 30-49 1.02 (0.86, 1.20) 50-69 1.00 ref 70-89 1.25 (1.05, 1.47) ≥90 1.22 (0.97, 1.53) Per 30 nmol/L increase 1.15 (1.04-1.27) Positive association between 25(OH)D & prostate cancer risk. No association with 25(OH)D collected in winter & spring but positive association with 25(OH)D collected in summer & autumn - men with 25(OH)D ≥ 90 vs 50-69 nmol/L had a 50% increased prostate cancer risk (RR=1.46; 95% CI, 1.07-2.00).
Albanes *et al* (2011) Finland	Men in ATBC study (n=1000 cases & 1000 controls) Median age (IQR): 57 (54-62) y	6.1 y (median)	*Median (IQR)* Cases: 34.5 (22.7-50) Controls: 33.6 (21.4-49.1)	OR (95% CI) 25(OH)D ≥ 75 vs < 25 nmol/L: 1.44 (0.91-2.30) OR (95% CI) 25(OH)D ≥ 75 vs 50-75 nmol/L: 1.16 (0.73-1.86) Increasing 25(OH)D concentration associated with greater prostate cancer risk.
Brandstedt *et al* (2012) Sweden	Men in the Malmö Diet & Cancer Study (n= 443 cases & 443 controls) Mean age (y) Cases: 61.7 (6.4)/Controls: 61.6 (6.4)	7.6 ± 3.5 y	Cases – 87.4 (26.4) Controls – 86.4 (27.3)	OR (95% CI) 25(OH)D ≤68 vs 69-84 nmol/L: 1.25 (0.95-1.65) OR (95% CI) 25(OH)D ≤68 vs 85-102 nmol/L: 1.37 (1.03-1.82) OR (95% CI) 25(OH)D ≤68 vs ≥ 103 nmol/L: 1.34 (0.99-1.82) Weak positive association between 25(OH)D and risk of prostate cancer.
Schenk *et al* (2014) USA	Men in the Prostate Cancer Prevention Trial (n=1695 cases & 1682 controls) Age: 55+ y	7 y	*Mean (95% CI)* White: 58.8 (28.6–120.9) African-American: 39.5 (15.7–99.7) Other: 49.1 (22.9–105.6)	OR (95% CI) 25(OH)D <44.7 vs >71.2 nmol/L: 1.18 (0.91-1.53) No association between 25(OH)D and prostate cancer risk.
Kristal *et al* (2014) USA	Men in the Selenium & Vitamin E Cancer Prevention Trial (n=1731 cases & 3203 controls) Mean age (y) Cases: 63.5 (±6.1)/Controls:63.3 (±6.5)	5-8y	69.2 (±28.8)	Hazard ratios (95% CI) for pre-specified 25(OH)D cutoffs (nmol/L): <37.5 vs ≥ 75: HR = 0.96 (0.75-1.23; p=0.76) Based on 25(OH)D distribution among cohort: <44.1 vs ≥ 90.7: HR = 0.88 (0.63-1.22; p=0.44) No significant association.
Shui *et al* (2012) USA	Men in the Health Professionals Follow up Study (n=1260 cases & 1331 controls) Mean age (y): 64.4 (7.8)	Median (IQR): 5.2 y (2.9-7.5)	Q1: 40.4 Q2: 56.7 Q3: 68.4 Q4: 89.1	Lethal prostate cancer OR (95% CI) 44.1 vs 89.1 nmol/L: 0.44 (0.24-0.79); p trend = 0.002 Overall prostate cancer OR (95% CI) 44.1 vs 89.1 nmol/L: 1.07 (0.86-1.34); p trend = 0.45 Highest vs lowest 25(OH)D quartile associated with significant reduction in lethal prostate cancer risk. No significant association with overall prostate cancer risk.
Oesophagus and stomach				
Abnet *et al* (2010) China, Finland, USA	Adults from 8 cohorts (Cohort Consortium Vitamin D Pooling Project of Rarer Cancers - VDPP) (n=1065 cases & 1066 controls) Median (IQR) age (y) Cases: 61 (55-67)/Controls: 61 (55-66)	Median (IQR): 5.3 y (2.4-9.1)	Median (IQR): Cases: 39.4 (26.3-56.1) Controls: 39.1 (25.8-56.7)	<25 vs 50 to <75 nmol/L: OR 0.90 (95%CI 0.65, 1.24) ≥100 vs 50 to <75 nmol/L: OR 0.81 (95%CI 0.39, 1.69) No association between 25(OH)D concentration and risk of upper GI cancer.

Study/Country	Population	Mean follow up	Mean 25(OH)D (nmol/L)	Results/comments
Larynx and oropharynx				
Arem *et al* (2011) Finland	Male smokers taking part in the ATBC study (n=340 cases & 340 controls) Median (IQR) age (y): 57 (53-61) y	20 y	Median (IQR): Cases 31 (21-47) Controls 32 (21-48)	For overall neck & shoulders: <25 vs 50 to < 75 nmol/L: OR = 0.96 (95% CI, 0.58-1.59) ≥75 vs 50 to < 75 nmol/L: OR = 1.35 (0.53-3.43) No association between 25(OH)D and head and neck cancer risk.
Lung				
Kilkkinen *et al* (2008) Finland	Cohort study (n=6937; 122 cases) Age: ≥ 30 y	24 y	42.9 (±19.6)	RR (95% CI) for highest (M > 52; F >47nmol/L) vs lowest tertile (M,35; F, 30nmol/L) of 25(OH)D: 0.72 (95% CI 0.43, 1.19): No association between 25(OH)D and lung cancer risk.
Weinstein *et al* (2011) Finland	Men in the ATBC study (n=500 cases & 500 controls) Median (IQR) age (y): 59 (55-62)	20 y	Median (IQR) Cases: 33.6 (20.7-50.2) Controls 35 (21.5-50.5)	25(OH)D < 25 vs 50-75 nmol/L: OR = 1.35 (95% CI, 0.87-2.10) 25(OH)D ≥ 75 vs 50-75 nmol/L: OR = 1.23 (95% CI, 0.64-2.36) No association between 25(OH)D and lung cancer risk.
Endometrium				
Zeleniuch-Jacquotte *et al (*2010)	Women from 7 cohorts (VDPP) (n=830 cases & 992 controls). Median (IQR) age (y): Cases: 8y (50-65)/Controls: 58 (50-64)	1.4-10.7 y	Median (IQR) Cases: 49.4 (34.6-66.4) Controls: 50.8 (36.7-67.1)	25(OH)D <25 vs 50 to <75 nmol/L: OR 1.02 (95% CI, 0.68-1.53) 25(OH)D ≥100 vs 50 to <75 nmol/L: OR 0.85 (95% CI, 0.47-1.53) No association between 25(OH)D and endometrial cancer.
Kidney				
Gallicchio *et al* (2010) USA	Adults from 8 cohorts (VDPP) (n= 775 cases & 775 controls) Median (IQR) age: 60 y (54-66)	Median (IQR): 5.5 y (2.7-9.9)	Median (IQR) Cases: 44.4 (30.7-62.9) Controls 45.4 (30.2-62.5)	25(OH)D <25 vs 50 to <75 nmol/L: OR=0.94 (95% CI, 0.64-1.37) 25(OH)D ≥100 vs 50 to <75 nmol/L: OR 0.92 (95% CI, 0.44-1.92) No association between 25(OH)D and kidney cancer.
Mondul *et al* (2014) Finland	Participants from the ATBC study (n=281 cases & 281 controls) Median (IQR) age (y): 57 (54-61)	17-20y	Winter/summer Q1: <19/ <29 Q2: 19-<29/29-<43 Q3: 29-<44/43-< 57 Q4: ≥44/ ≥57	25(OH)D Q4 vs Q1: OR = 1.45 (95% CI, 0.86-2.44) No association between 25(OH)D and kidney cancer.
Non-Hodgkin lymphoma				
Purdue *et al* (2010) USA & China	Participants pooled from 10 cohorts (VDPP) (n=1353 cases & 1778 controls) Median (IQR) age (y) Cases: 62 (56-68)/Controls: 61 (55-67)	Median (IQR): 5.2 y (2.5-8.7)	Median (IQR) Cases 55.7 (40.6-70.1) Controls: 53.5 (38.8-68.8)	25(OH)D <25 vs 50 to <75 nmol/L: OR=1.08 (95% CI, 0.78-1.50) 25(OH)D ≥100 vs 50 to <75 nmol/L: OR 0.86 (95% CI, 0.57-1.27) No association between 25(OH)D concentration and non-Hodgkin lymphoma.
Hepatocellular cancer				
Fedirko *et al* 2014 10 European countries	Adults in the European Prospective Investigation into Cancer and Nutrition (EPIC) (n=138 cases & 138 controls) Mean age: 59.9 (3)	6 y (3.4)	Mean (5^{th} -95^{th} percentile) Cases: 41.7 (16.9-82.3) Controls: 49.9 (24.8-90.9) Mean (SD) by tertiles T1: 33.5 (7.7), T2: 51.3 (4.4), T3: 73.6 (13.7)	Incident Rate Ratio (95% CI) 25(OH)D tertile 3 vs 1: IRR = 0.51 (0.26-0.99) 25(OH)D ≥75 vs < 50 nmol/L: IRR 0.73 (95%CI 0.25, 2.13) Per 10 nmol/L increase in 25(OH)D: IRR = 0.80 0.68-0.94) Higher 25(OH)D associated with a lower risk of liver cancer.
Wang *et al* (2013) China	Adults from 2 trials (incl. 20µg/d vit D) (n=226 cases & 1063 controls) Median (IQR) age (y) Cases: 55 (49-61)/Controls:55 (50-61)	Over 22 y	Median (IQR) Cases: 20 (14-28.6) Controls 20.1 (13.7-30.3)	Women, Q1-Q4 (nmol/L): <11.7, ≥11.7 to <14.86, ≥14.86 to <21.8, ≥21.8 Men, Q1-Q4 (nmol/L): <18.1, ≥18.1 to <25.3, ≥25.3 to <38.8, ≥38.8 Q4 vs Q1: OR = 0.74 (95% CI, 0.47-1.18) No significant association between 25(OH)D &risk of liver cancer incidence.

Study/Country	Population	Mean follow up	Mean 25(OH)D (nmol/L)	Results/comments
Bladder cancer				
Mondul *et al* (2010) Finland	Men in the ATBC study (n=250 cases & 250 controls). Median (IQR) age (y) Cases: 59 (55-63)/Controls: 59y(56-63)	20 y	Not reported.	OR (95% CI) 25(OH)D <25 vs. ≥ 50 nmol/L = 1.73 (1.03-2.91) Significant inverse association between serum 25(OH)D and bladder cancer risk.
Mondul *et al* (2012) US	Adults in the PLCO study (375 cases & 375 controls). Median (IQR) age (y) Cases: 64 (60-68)/Controls 64 (61-67)	13 y	Median (IQR) Cases 52.4 (39.6-65.8) Controls 53.6 (39.5-68.1)	25(OH)D <25 vs 50 to <75 nmol/L: OR=0.74 (95% CI, 0.29-1.87) 25(OH)D ≥75 vs 50 to <75 nmol/L: OR 0.85 (95%CI 0.53, 1.38) No significant association between 25(OH)D and bladder cancer risk.
Melanoma				
Major *et al* (2012) Finland	Men in ATBC study n=92 cases & 276 controls) Median (IQR) age (y): 57.5 (53.5-6)	Median Cases: 8.9 y Controls: 18.2 y	Median (IQR): Cases: 33.1 (21.9-51.6) Controls: 31.8 (20.9-48.5)	25(OH)D <25 vs ≥ 50 nmol/L: OR=1.32 (95% CI, 0.64-2.72) No association between serum 25(OH)D &melanoma risk.
Afzal *et al* (2013) Denmark	Participants in the Copenhagen City Heart Study (n=10,060; 78 cases)	28 y	Median: 41 Median among those who later developed melanoma: 51	25(OH)D ≥50 vs <25 nmol/L: HR=4.7 (95% CI, 0.96-23.3) 25(OH)D >67th vs ≥34th percentile: HR=6.31 (95% CI, 1.38-28.8) Per 10 nmol/L increase in 25(OH)D: HR=1.45 (95% CI 1.22-1.73) Increasing 25(OH)D associated with increased risk of melanoma skin cancer.
Van der Pols *et al* (2013) Australia	Adults in subtropical community (n=1191). Mean age: 54 y	11 y	Not reported.	Basal cell carcinoma (300 cases): 25(OH)D <75 vs ≥75 nmol/L: OR=1.51 (95% CI, 1.10-2.07) Squamous cell carcinoma (132 cases): 25(OH)D <75 vs ≥75 nmol/L: OR, 0.67 (95% CI, 0.44-1.03) Melanoma (17 cases): 25(OH)D <75 vs ≥75 nmol/L: OR=2.71 (95% CI, 0.98-7.48) 25(OH)D > 75 nmol/L associated with increased risk of basal cell carcinoma & melanoma
Pancreatic cancer				
Stolzenberg-Solomon *et al* (2010) USA & China	Participants from 8 cohort studies (VDPP) (n=952 cases & 1333 controls) Median (IQR) age (y): Cases:62y (56-68)/Controls: 62 (57-67)	Median 6.5 years	Median (IQR) Cases: 49.3 (2.0-156) Controls: 50.8 (2.6-127.2)	25(OH)D ≥100 vs <25 nmol/L: OR=2.24 (95% CI, 1.22-4.12) 25(OH)D ≥100 vs < 50 to <75 nmol/L: OR=2.12 (95% CI, 1.23-3.64) Significant increase in pancreatic cancer risk with higher compared to lower 25(OH)D .

Table 40: Meta-analyses of RCTs and prospective studies of vitamin D supplementation/25(OH)D concentration on CVD risk

Study	Methods	Results	Conclusions
RCTs			
Wang *et al* (2010)	Selection criteria: *Inclusion*: RCTs & prospective studies. *Exclusion*: Case reports, ecological, cross-sectional & retrospective case-control studies. Studies that did not assess vitamin D/calcium supplement use or compare between recipients & non-recipients of vitamin D, calcium or a combination of supplements.	RCTs: Vit D alone: 2 RCTs, 25 µg/d to 2,500µg/4 m RR = 0.90 (95% CI, 0.77-1.05) Vitamin D & calcium: 2 RCTs, 10 µg/d to 20 µg/d RR = 1.04 (95% CI, 0.92-1.18)	Slight significant reduction in CVD risk with vitamin D vs placebo. No significant reduction in CVD risk with vitamin D + calcium vs placebo.
Bolland *et al* (2011)	Selection criteria: *Inclusion:* Trials comparing co-administered calcium and vitamin D with placebo. *Exclusion:* not reported.	Calcium and vitamin D supplements and CVD events: 3 trials (n=10,090) 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 Myocardial infarction or stroke: RR = 1.16 (95% CI, 1.02-1.32) p=0.02	Vitamin D & calcium supplementation increased risk of myocardial infarction & stroke.
Ford *et al* (2014)	Selection criteria: *Inclusion:* RCTs with adults ≥60y & ≥1 y follow up; any vit D/analogue intervention; coadministration with other treatment; if comparator group received same treatment. *Exclusion:* Studies in which participants selected solely on the basis of renal impairment, steroid induced osteoporosis, or psoriasis.	21 RCTs (n=13,033) Cardiac failure: HR = 0.82 (95% CI, 0.58-1.15) Myocardial infarction: HR = 0.96 (95% CI, 0.83-1.10) Stroke: HR = 1.07 (95% CI, 0.91-1.29)	Vitamin D supplementation may be protective for heart failure but not myocardial infarction or stroke.
Prospective studies			
Grandini *et al* (2010)	Selection criteria: *Inclusion*: Cohort studies reporting association between initial 25(OH)D concentration with cardiovascular event incidence or mortality. Studies investigating incident fatal or non-fatal myocardial infarction, acute coronary syndrome, coronary heart disease (CHD) or combined outcomes of cardiovascular & cerebrovascular diseases (stroke). *Exclusion:* Not in English; study population selected according to presence of a disease other than CVD. Exposure & disease status determined simultaneously. Studies which analysed clinical outcomes of peripheral arterial disease, congestive heart failure, atherosclerosis or stroke were included only if those outcomes were analysed in combination with CHD endpoints.	CVD incidence: 3 cohort studies & 1 nested case control study (n=5,253; mean age 59-79 y; follow up 5-10 y). RR 1.54 (95%CI 1.22, 1.95) p=0.47 CVD mortality: 5 cohort studies (n=24,387; mean age 45-75 y, follow up 6.2-27.1 y) RR 1.83 (95%CI 1.19, 2.79) p = 0.006	Inverse association between baseline 25(OH)D and cardiovascular risk suggested.
Wang *et al* (2012)	Selection criteria: *Inclusion:* prospective studies on association between 25(OH)D at baseline & CVD risk. *Exclusion:* Ecological, cross-sectional or case control studies, case reports. Studies without 25(OH)D measurement or ascertainment of major clinical CVD events. Studies that did not compare CVD event rates between different 25(OH)D concentrations and studies of participants selected by confirmed medical conditions.	19 studies (n=65,994). Lowest vs highest 25(OH)D): Total CVD: RR = 1.52 (95% CI, 1.30-1.77) p=0.57 CVD mortality: RR 1.42 (95%CI 1.19, 1.71) CHD: RR 1.38 (95%CI 1.21, 1.57) Stroke: RR 1.64 (95%CI 1.27, 2.10)	Significant inverse association between 25(OH)D 20-60 nmol/L and CVD risk.

Table 41: Prospective studies on 25(OH)D concentration and CVD risk

Study/country	Population description	Mean Follow up	Mean 25(OH)D (nmol/L)	Results/ comments
Messenger *et al* (2012) USA	Men participating in the Osteoporotic Fractures in Men study (MrOS) (n=821) Mean age (SD) in those with: CVD event, 77.4 (5.5)/No CVD event,76.1 (5.6)	4.4 y (median)	Median (IQR): CVD event 62.7 (48.9 – 74.1) No CVD event 63.4 (50.9-75.1)	25(OH)D (nmol/L) lowest (12.2-50.2) vs highest (75.3-138.3) Q1 HR =1.18 (95% CI, 0.69-2.03); p=0.85 No significant association between 25(OH)D & CVD outcomes.
Welsh *et al* (2012) West Scotland	Participants in the MIDSPAN Family Study (n=2338) Age: 45-64y	14.4 y (median)	46.4 (median)	25(OH)D <37.5 nmol/L: HR = 1.00 (95% CI, 0.77-1.31) No association between 25(OH)D & CVD.
Karakas *et al* (2013) Germany	Adults in the MONICA/KORAstudy (n=1783) Age: 35-74y	11 y	Men CHD cases 37.7 (1.03) Non cases 43.9 (1.02) Women CHD cases 31.9 (1.05) Non cases 39.7 (1.01)	Men: 25(OH)D 66.9 (54.14-153.92) vs 27 (5.08-35.02) nmol/L: HR= 0.84 (95% CI, 0.52-1.35); p=0.461 Women: 25(OH)D 58.5 (47.7-127.7) vs 26.4 (9.9-33.2) nmol/L: HR 0.42 (95%CI 0.19, 0.93); p=0.028 25(OH)D >47.7 nmol/L associated with decreased CHD risk in women ; no significant association found in men.
Robinson *et al* (2013) USA	Adults in the Multi-Ethnic Study of Atherosclerosis (MESA) Mean age (y): 62	Median (IQR): 8.5 (7.6-8.6))	63.7 (26.5)	*All:* 25(OH)D <85.92 vs ≥124.58 nmol: OR=1.32 (95% CI, 0.95-1.83) per 42.95 decrement *All*: OR=1.15 (95% CI, 1.01-1.32); p=0.04 *White:* OR=1.26 (95% CI 1.06-1.49) p=0.008 *Chinese:* OR=1.67 (95% CI, 1.07-2.61) p=0.03 *Black:* OR=0.93 (95% CI, 0.73-1.20) p=0.59 *Hispanic:* OR=1.01 (95% CI, 0.77, 1.33) p=0.95 Inverse association between 25(OH)D & CHD risk in white & Chinese adults but not among black & Hispanic participants.
Perna *et al* (2013) Germany	Participants in the ESTHER cohort study (n=7709) Age (y): 50-74	6.5 y	14.5% < 30 44.5% 30 to <50 41.1% ≥ 50	*Total CVD* <30 vs ≥50 nmol/L: OR=1.24 (95% CI, 1.02-1.50) *per* 25 nmol/L: OR 0.95 (95% CI, 0.89-1.01) CVD risk Increased at 25(OH)D concentrations < 75 nmol/L.
Kuhn *et al* (2013) Germany	Participants in EPIC-Germany cohort (n=2132) Mean age (y) – 50.6 ± 8.7	7.6 y	47.2 (±18.3)	HR for CVD risk according to quartiles of 25(OH)D: 28.9 vs 66.5 nmol/L: HR=1.19 (95%CI, 0.93-1.52); p=0.12 HR for CVD risk according to predefined 25(OH)D categories: <25 vs ≥ 50 nmol/L: HR=1.53 (95%CI, 1.12-2.09); p<0.01 25(OH)D < 25 nmol/L significantly increased CVD risk

Table 42: Meta-analyses of RCTs and prospective studies of vitamin D supplementation/25(OH)D concentration on hypertension risk

Study	Methods	Results	Conclusions
RCTs			
Witham *et al* (2009)	Selection criteria: *Inclusion:* RCTs comparing vitamin D with placebo (included studies that used UVB light to produce an increase in 25(OH)D). *Exclusion:* Not reported..	11 RCTs (n=716; mean age, 51-75y; 5 wks – 12 m) Studies with elevated mean baseline BP (>140/90 mmHg) (8 studies; n=545) Mean change in BP (intervention vs placebo) SBP - 3.6 mmHg (95% CI, 8.0-0.7) p=0.1 DBP - 3.1mmHg (95% CI, -5.5 to -0.6) p=0.01 Studies with normal mean baseline BP (<140 mmHg) (3 studies; n=255) 3 studies; none showed effect on BP.	Small significant reduction in diastolic but not systolic BP in hypertensive patients..
Wu *et al* (2010)	Selection criteria: *Inclusion:* RCTs reporting baseline & follow up BP. *Exclusion:* Uncontrolled trials, observational & animal studies; studies in patients with kidney diseases, hypercalcemia, diabetes, or impaired glucose tolerance; pregnant women & children..	4 RCTs (n=429; mean age, 64 y) Mean change in BP (intervention vs placebo) SBP -2.44 mmHg (95% CI, -4.86 to -0.02) DBP -0.02mmHg (95% CI to -2.19 to 1.94)	Significant reduction in systolic but not diastolic BP.
Kunutsor *et al* (2014)	Selection criteria: *Inclusion:* RCTs on effects of vitamin D supplementation alone. *Exclusion:* Studies with calcitriol or one of its analogues.	SBP: 16 trials (20-214 µg/d) Mean change -0.94 mmHg (95% CI, -2.98 to 1.10) DBP: 15 trials (20-214 µg/d) Mean change -0.52 mmHg (95% CI, -1.18 to 0.14)	No association.
Beveridge *et al* (2015)	Selection criteria: *Inclusion:* BP or other measures of vascular function as outcomes; any baseline 25(OH)D concentration; BP reduction or changes in surrogate markers of cardiovascular risk; minimum 4 weeks; vitamin D2, D3, calcitriol, 1-α – hydroxylated version of vitamin D, paricalcitol and doxerocalciferol. *Exclusion:* Participants younger than 16 y or patients who were receiving dialysis.	52 RCTs; 46 included in trial-level meta-analysis; 27 included in individual patient data analysis. *Effect size* Trial level data SBP: 0.0 (95% CI, -0.8 – 0.8) mm Hg (p=0.97; I^2 =27%) DBP: -0.1 (95% CI, -0.6 – 0.5) mm Hg (p=0.84; I^2=20%) Individual patient data SBP: -0.5 (95% CI, -1.3 – 0.4) mm Hg (p=0.27; I^2 =0%) DBP: 0.2 (95% CI, -0.3 – 0.7) mm Hg (p=0.38; I^2=0%)	No effect of vitamin D supplementation on BP.
RCTs & Prospective studies			
Pittas *et al* (2010)	Selection criteria: *Inclusion:* Studies in English language & in healthy populations. RCTs of vit D supplementation ± calcium. Nested case control studies. *Exclusion:* Cross-sectional, retrospective cohort & case control studies and RCTs <1month). Studies in children, pregnant women, & patients with conditions affecting vitamin D metabolism (e.g. chronic kidney disease); trials using vit D analogues.	RCTs: 9 trials (n=37,162; 10-214 µg/d) Systolic BP: Weighted mean difference -1.9 mmHg (95% CI -4.2 to 0.4) Diastolic BP: Weighted mean difference -0.1 mm Hg (95% CI -0.7 to 0.5) Cohort: 2 studies (3 cohorts; n=3,295; follow up, 7-8 y) 25(OH)D Lowest (<37-51 nmol/L) vs highest (>75-81 nmol/L): RR = 1.76 (95% CI, 1.27-2.44)	RCTs - No statistically significant effect of vitamin D supplementation vs placebo on systolic or diastolic BP. Cohort studies: 25(OH)D <37-51 nmol/L associated with increased hypertension risk.
Burgaz *et al* (2011)	Selection criteria: *Inclusion:* Cohort, case-control or cross-sectional studies with 25(OH)D as exposure and results reported as RR/OR (95% CI). *Exclusion:* None reported.	18 studies (4 prospective, 14 cross-sectional; n=78,028; age range, 18-96y) Highest vs lowest 25(OH)D concentration (16 studies): OR=0.73 (95% CI, 0.63-0.84; p=0.007) (I^2=32%; p=0.007) For each 40 nmol/L increase in 25(OH)D (17 studies): OR=0.84 (95% CI 0.78-0.90) p=0.000	25(OH)D concentration inversely associated with hypertension.

Table 43: Prospective studies of 25(OH)D concentration and hypertension risk

Study/country	Population description	Mean Follow up	Mean 25(OH)D (nmol/L) at baseline	Results/ comments
Wang *et al* (2013) USA	Normotensive men (n=1,211); mean age, 57y	15.3 y	<u>Men who susbsequently developed hypertension</u> Winter/spring: 57.9 (20.7) (n=59) Summer/fall: 74.9 (27.5) (n=308) <u>Men who did not develop hypertension</u> 55.9 (22.5) (n=58) 77.4 (26.2) (n=235) No significant difference in baseline 25(OH)D between men who developed hypertension & those who did not.	Risk of incident hypertension according to 25(OH)D concentration Hazard ratios (95% CI) Q1: ref Q2 0.94 (0.69-1.27) Q3 0.69 (0.50-0.96) Q2 0.82 (0.60-1.13) (p trend: 0.43)

All-cause mortality

Table 44: Systematic reviews of RCTs and prospective studies of vitamin D supplementation/25(OH)D concentration on all-cause mortality risk

Study	Methods	Results	Author's conclusions
Bjelakovic *et al* (2014)	Selection criteria: *Inclusion*: RCTs of vitamin D (any type & any route) vs placebo/no intervention in the general population or patients in stable phase of disease. *Exclusion:* Patients with secondary induced osteoporosis or cancer, pregnant &lactating women.	56 trials (n=95,286); age range, 18-107 y; mean duration, 4.4 y). All trials RR = 0.97 (95% CI, 0.94-0.99) p=0.02 Vitamin D3 alone vs placebo/no intervention (13 trials, n=12609) RR = 0.92 (95% CI, 0.85-1.00) p=0.06; ; I^2=0% Vitamin D3 + Ca vs placebo/no intervention (27 trials, n=63051) RR = 0.96 (95% CI, 0.92-0.99) p=0.03); Vitamin D2 alone vs placebo/no intervention (8 trials; n=17079) RR = 1.03 (95% CI, 0.96-1.12) p=0.37 ; I^2=14% Vitamin D2 + Ca vs placebo/no intervention (5 trials; n=1307) RR = 1.0 (95% CI, 0.64-1.57) p=1.00 ; I^2=11% Trials (n=45) which recruited participants on basis of 25(OH)D concentration: 25(OH)D < 50 nmol/L (26 trials): RR=0.95 (95% CI, 0.91-0.99) p=0.01; I^2=0% 25(OH)D ≥ 50 nmol/L (19 trials): RR=0.95 (95 CI, 0.87-1.05); P=0.30; I^2=0%	Overall vitamin D supplementation decreased mortality. In analysis of the different types of vitamin D supplementation (D2 and D3) given without calcium there was no significant effect on mortality.
Chowdhury *et al* (2014)	Selection criteria: *Inclusion:* Cohort studies on association of 25(OH)D with cause specific or all cause deaths in healthy adults. Randomised intervention studies which assessed effects of vit D supplements alone with placebo/no treatment & collected cause specific or all-cause mortality endpoints. *Exclusion:* no relevant exposure of outcome data, reviews, letter or editorials, case reports, in vitro/functional studies, non-adult population, inappropriate baseline population, non-randomised trials.	*Cohort studies:* Bottom vs top thirds of baseline 25(OH)D 27 primary prevention cohorts (n=780,990); RR = 1.35 (95% CI, 1.22- 1.49) 41 secondary prevention cohorts (n=59,918); RR = 1.50 (95% CI, 1.36-1.65) All cohorts (n=840,908); RR = 1.44 (95% CI, 1.34-1.55) Association of 25(OH)D (nmol/L) with all cause mortality (based on 1° prevention cohorts) 52-72 vs ≥ 75: RR = 1.07 (95% CI, 1.01–1.15) 25-50 vs ≥ 75: RR = 1.20 (95% CI, 1.12–1.27) <25 vs ≥ 75: RR = 1.50 (95% CI, 1.21–1.87 For each 25 nmol/L increase in 25(OH)D: RR = 1.16 (95% CI, 1.08-1.23) *RCTs* (22 RCTs; n=30,716) Vitamin D3 (14 RCTs): RR = 0.89 (95% CI, 0.80-0.99) Vitamin D2 (8 RCTs): RR = 1.04 (95% CI, 0.97-1.11)	Observational studies indicate inverse association between 25(OH)D & all cause mortality risk. In all RCTs combined, vit D did not reduce overall mortality. When stratified by type, vitamin D3 significantly reduced all cause mortality in older adults.
Schottker *et al* (2014)	Meta-analysis of individual participant data Selection criteria: *Inclusion*: Cohort studies with participants aged 50-79 y *Exclusion*: Smokers, participants without 25(OH)D measurement, missing values for covariates used in main model, or lost to follow up due to unknown reasons.	8 cohort studies from USA and Europe (n=26,018), age range, 50-79 y, median follow-up, 4.2-15.8 y; median 25(OH)D, 24-62 nmol/L. Highest vs lowest quintile of 25(OH)D RR 1.57 (95% CI, 1.36-1.81)	Increased risk of all-cause mortality in the lowest quintile compared to the top quintile of 25(OH)D concentration

Table 45: Prospective studies on serum 25(OH)D concentration and all-cause mortality risk

Study	Population	Mean follow up	Mean 25(OH)D (nmol/L)	Results/ comments
Formiga *et al* (2014) Spain	Community dwelling older adults (n=328) Age: >85 y	median 2.8 y ()	70/L (±75) Q1: <35 Q2: 35-61.7 Q3: 62-83.4 Q4: >84	Q1: OR = 1.28 (95% CI, 0.61-2.6); p=0.41 Q2: OR = 1.36 (95% CI, 0.67-2.74) Q3: OR = 0.76 (95% CI, 0.34-1.68) Q4: OR 1.00 (reference) 25(OH)D not associated with overall mortality in older community dwelling adults.
Wong *et al* (2013) Australia	Participants from the Health in Men Study (HIMS) (n=4203) Mean age 76 y (70-88)	6.7 y	68.3 (±23.3) Q1 10-52.8 Q2 52.9-67.3 Q3 67.4-81.6 Q4 81.7-238.4	Per 10 nmol/L decrease in 25(OH)D: OR = 1.04 (95% CI, 1.01-1.07) Halving of 25(OH)D: OR = 1.21 (95% CI, 1.08-1.35) By quartile: Q1: OR = 1.20 (95% CI, 1.02-1.42) Q2: OR = 1.0 (ref) Q3: OR = 0.99 (95% CI, 0.84-1.17) Q4: OR = 0.99 (95% CI, 0.83, 1.17)
Sempos *et al* (2013) US	Participants in NHANES III study (n=15,099) Age: 45 years	15 y	64 (±0.73)	<20 nmol/L: RR = 1.6 (95% CI, 1.2-2.2) 20-29 nmol/L: RR = 1.5 (95% CI, 1.2-1.8) 30-39 nmol/L: RR = 1.3 (95% CI, 1.1-1.5) 40-49 nmol/L: RR = 1.1 (95% CI, 0.96-1.3) 50-59 nmol/L: RR = 1.2 (95% CI, 1.01-1.3) 60-74 nmol/L: RR = 1.1 (95% CI, 0.99-1.3) 75-99 nmol/L: RR 1.0 (ref) 100-119 nmol/L: RR = 1.1 (95% CI, 0.9-1.4) ≥120 nmol/L: RR = 1.4 (95% CI, 0.9-2.2) Reverse J shaped association between 25(OH)D and all cause mortality..

Table 46: Systematic reviews of intervention & observational studies on vitamin D supplementation/25(OH)D concentration and autoimmune disease risk

Study	Methods	Results	Author's conclusions
Antico *et al* (2012)	Selection criteria: *Inclusion*: ecologic studies correlating 25(OH)D with risk of developing autoimmune disease (AID) in different geographical areas; prospective studies correlating 25(OH)D with risk of AID, studies relating administration of vit D & risk of AID clinical studies of incidence & prevalence of AID in relation to 25(OH)D concentration. *Exclusion:* Not reported.	*25(OH)D concentration and AID risk* 2 prospective studies correlating serum 25(OH)D with AID – no association was found between 25(OH)D & RA risk in 1 study; in the other study, higher 25(OH)D concentrations were associated with significantly lower risk of multiple sclerosis but only among whites (not black or Hispanic individuals) *Vitamin D supplementation and AID risk* Main outcome measure was risk of type 1 diabetes in infancy 9 observational studies (case-control & cohort) OR = 0.71 (95% CI, 0.60-0.84)	Only studies of type 1 diabetes suggest risk significantly reduced in infants treated with vitamin D.
Harvey *et al* (2014)	Selection criteria: *Inclusion:* observational studies (case-control, cohort, cross-sectional), intervention studies. *Exclusion:* studies not in English, did not measure maternal 25(OH)D in or immediately after pregnancy or supplement participants with vitamin D in pregnancy, or where an outcome of interest was not measured. Systematic reviews.	*Offspring asthma and atopy* - 10 observational studies. 5 found significantly reduced risk of offspring asthma or atopy with higher maternal 25(OH)D; 3 found a significant positive association between maternal 25(OH)D & offspring risk of asthma or atopy; 2 studies found no significant association between late pregnancy 25(OH)D & offspring lung function at 6-7y. No intervention studies were identified. *Offspring Type 1 diabetes mellitus* -3 observational studies. 1found a significantly increased risk of type 1 diabetes in the offspring with lower maternal 25(OH)D concentration in late pregnancy. The remaining studies found no significant relationship. No intervention studies were identified.	Substantial heterogeneity in terms of study design, outcome definition and exposure definition with a variety of conflicting results. Difficult to conclude any definitive relationship between maternal 25(OH)D & offspring asthma & atopy. The studies relating maternal 25(OH)D to risk of offspring type I diabetes were generally consistent in suggesting an inverse relationship. However one used vitamin D dietary intake.

Table 47: Meta-analysis of genetic studies on multiple sclerosis risk

Study	Methods	Results	Author's conclusions
Huang & Xie (2012)	Selection criteria: *Inclusion*: Studies that evaluated VDR polymorphisms & risk of MS; case-control design based on unrelated individuals; and sufficient data for genotype distributions in both cases and controls. *Exclusion*: No control; no usable data reported	11 case-control studies (2599 cases & 2816 controls). 2 studies in Asian & 9 in white populations. *Apa-I polymorphism (rs7975232)* 4 studies (599 cases & 878 controls) Dominant model: OR=0.71 (95% CI, 0.46-1.12); p=0.14 Recessive model: OR=1.09 (95% CI, 0.85-1.38); p=0.51 Homozygote model: OR=0.72 (95% CI, 0.39-1.36); p=0.32 *Bsm-I polymorphism (rs1544410)* 3 studies (352 cases & 582 controls) Dominant model: OR=0.81 (95% CI, 0.36-1.80); p=0.60 Recessive model: OR=1.17 (95% CI, 0.83-1.64); p=0.38 Homozygote model: OR=1.48 (95% CI, 0.90-2.45); p=0.12 *Fok-I polymorphism (rs10735810)* 6 studies (1775 cases &1830 controls) Dominant model: OR=0.99 (95% CI, 0.87-1.14); p=0.93 Recessive model: OR=0.89 (95% CI, 0.74-1.07); p=0.21 Homozygote model: OR=0.90 (95% CI, 0.74-1.11); p=0.33 *Taq-I polymorphism (rs731236)* 8 studies (2472 cases & 2446 controls) Dominant model: OR=1.12 (95% CI, 1.00-1.26); p=0.06 Recessive model: OR=1.03 (95% CI, 0.88-1.20); p=0.74 Homozygote model: OR=1.04 (95% CI, 0.78-1.38); p=0.80	The VDR Apa-I, BSM-I, Fok-I and Taq-I polymorphisms not associated with MS risk.
Tizaoui *et al* (2014)	Selection criteria: *Inclusion:* Case control studies that reported the number of individual genotypes and/or alleles for VDR polymorphisms in cases and controls. Each study had disease outcome definitions that followed accepted diagnostic guidelines. *Exclusion:* Cross-sectional studies, size of cases and controls not reported. Outcome measure The association of VDR polymorphisms with MS risk.	13 case-control studies (n=3300 cases & 3194 controls) TaqI polymorphism: 11 studies (3011 cases & 2810 controls) Homozygous model: OR=0.90 (95% CI, 0.78-1.04); p=0.163 BsmI polymorphisms: 3 studies (247 cases & 276 controls) Codominant model: OR=0.85 (95% CI, 0.27-2.66) Allele contrast model: OR=0.91 (95%CI, 0.51-1.64); p=0.771 ApaI polymorphism: 5 studies (858 cases & 1097 controls) Codominant model: OR1=0.83 (95% CI, 0.64-1.08); p=0.016 OR2=1.34 (95% CI, 0.85-2.27); p=0.19 OR3=1.45 (95% CI, 1.04-2.2.08); p=0.03 FokI polymorphism: 7 studies (1989 cases & 1872 controls) Recessive model: OR=1.03 (95% CI, 0.86-1.24); p=0.074	No significant association between TaqI and BsmI polymorphisms and multiple sclerosis risk Significant association between the AA ApaI & FF FokI polymorphisms and multiple sclerosis.

Table 48: Intervention studies on vitamin D supplementation and risk of asthma & rheumatoid arthritis

Study/country	Study population	Intervention	Baseline 25(OH)D	Post intervention 25(OH)D	Results	Comments
Asthma						
Goldring *et al* (2013) UK	Pregnant women at 27 weeks gestation (n=180)	1. Vitamin D2 (20 µg/d) 2. Vitamin D3 (5000 µg –single dose) 3. No vitamin D	%. <25nmol/L 1. 48% 2. 45% 3. 42%	Child 25(OH)D at age 3 years (median) 1. 32nmol/l (21-66) 2. 42nmol/l (30-93) 3. 42nmol/l (27-68)	HR =0.86 (95% CI, 0.49-1.50); p=0.69	No significant difference in risk of wheeze between treatment groups. Also no difference between groups in prevalence of eczema or atopy.
Rheumatoid arthritis						
Racovan *et al* (2011) US	Participants in Women's Health Initiative (n=36,282) Age: 50-79 years	1. D3 (10 µg/d) & Ca (100 mg/d) 2. Placebo 5.1 years follow up	Not reported.	Not reported.	HR 1.04 (95%CI 0.76, 1.41)	No significant difference in cases of rheumatoid arthritis between groups.

Table 49: Observational studies on serum 25(OH)D concentration and risk of autoimmune disease/allergic disorders

Reference/Country	Population	Follow-up (y)	Mean baseline 25(OH)D (nmol/l)	Results	Comments
Asthma & atopy					
Tolppanen *et al* (2013) UK	Children in the AVON Longitudinal Study of Parents and Children Wheezing (n=3323) Asthma (n=3323) Mean age: 9.8 y	9.8	Not reported	**25(OH)D2** Wheezing: OR=0.83 (95% CI, 0.68-1.00) Asthma: OR=0.89 (95% CI, 0.78-1.02) **25(OH)D3** Wheezing: OR=1.14 (95% CI, 1.03-1.28) Asthma: OR=1.02 (95% CI, 0.93-1.12)	25(OH)D2 concentration inversely associated with flexural dermatitis and wheezing. 25(OH)D3 was positively associated with wheezing.
Mai *et al* (2012) Norway	Adults in population health survey (n=2542) Mean age (y) Male cases: 40.3 Male controls: 40y Female cases: 39.1 Female controls: 39.7	11	*Female* Cases: 56.7 (±23.7) Controls: 59.5 (±23.1) *Male* Cases: 54.8 (±20.8) Controls: 58.9 (±23.5)	Women 25(OH)D <50 nmol/L: OR=0.94 (95% CI, 0.67-1.32) 25(OH)D 50-74.9 nmol/L: OR=0.80 (95% CI, 0.57-1.13) ≥75 nmol/L: OR 1.00 (ref) *per 25 nmol/L reduction:* OR=0.97 (95% CI,0.85-1.12) Men <50 nmol/L: OR=1.14 (95% CI, 0.94-1.37) 50-74.9 nmol/L: OR=1.50 (95% CI, 0.95-2.38) ≥75 nmol/L: OR=1.00 (ref) *per 25 nmol/L reduction:* AOR 1.14 (0.94, 1.37)	No significant association between baseline 25(OH)D concentration and asthma.
Hollams *et al* (2011) Australia	Children from community birth cohort (n=1380).	14	At age 14 y <50 4.4% 75 59.3% 50-75 36.3%	Asthma OR=0.11 (95% CI, 0.02-0.84); p=0.03 Atopy OR=0.14 (95% CI, 0.04-0.47) p=0.002	25(OH)D concentration inversely associated with developing atopy and asthma at 14 y.
Jones *et al* (2012) Australia	Infants in an Australian birth cohort at high risk of developing allergy (i.e. at least 1 parent with family history of allergic disease) (n=231)	1	Mean cord blood 25(OH)D3: 58.4 (± 24.1)	Eczema*:* *p*er 10 nmol/L increase in 25(OH)D: OR=0.86 (95% CI, 0.739-0.995); p=0.04	Eczema affected 34% of intants in study.
Chawes *et al* 2014 Denmark	Birth cohort of children born to mothers with physician verified asthma (n=257)	7	Median: 47.6 (10-145)	Asthma < 50 vs >75 nmol/L: HR=1.63 (95% CI, 0.47-5.6); p=0.38 Eczema: < 50 vs >75 nmol/L: HR=2.0 (95% CI, 0.59-6.76); p=0.94	Cord blood 25(OH)D concentration had no association with asthma or eczema at 7 y.

Reference/Country	Population	Follow-up (y)	Mean baseline 25(OH)D (nmol/l)	Results	Comments
Rothers *et al* (2011) US	Children from prospective birth cohort study of immune system (n=219)	5	Median (IQR) cord blood: 64 (49-81	Allergic Rhinitis 25(OH)D <50 nmol/L: OR=1.1 (95% CI, 0.4-2.9); p=0.81 25(OH)D 50-74.9 nmol/L:OR=1.00 (ref) 25(OH)D 75-99.9 nmol/L: OR=0.6 (95% CI, 0.2-1.8); p=0.38 Asthma (n=194) 25(OH)D <50 nmol/L: OR=0.5 (95% CI, 0.2-1.6); p=0.26 25(OH)D 50-74.9 nmol/L:OR=1.00 (ref) 25(OH)D 75-99.9 nmol/L: OR=1.1 (95% CI, 0.4-3.1); p=0.84	No significant association between 25(OH)D concentrations and allergic rhinitis or asthma.
Weisse *et al* (2013) Germany	Mother and child pairs from cohort investigating influence of lifestyle and environment factors on newborn allergy risk (n= 378 mother child pairs)	2	Median (IQR(Maternal, 34 wks - 55.4 (35.9-77.9) Cord blood – 27.3 (17.5-43.4)	<u>Eczema</u>: Association with maternal 25(OH)D (80-152 vs 15-36 nmol/L) *1st year of life*: OR=1.16 (95% CI,0.79-1.71) p=0.45 *2nd year of life*: OR=1.13 (95% CI, 0.74-1.72) p=0.06 *2 year lifetime:* OR=1.14 (95% CI, 0.81-1.61) p=0.44 Association with cord blood 25(OH)D (43.4 vs 3.7-17.4 nmol/L) *1st year of life:* OR=1.17 (95% CI, 0.76-1.81) p=0.81 *2nd year of life:* OR=1.26 (95% CI, 0.78-2.02) p=0.34 *2 year lifetime:* OR=1.20 (95% CI, 0.81-1.76) p=0.37 <u>Food allergy:</u> Association with maternal 25(OH)D (80-152 vs 15-36 nmol/L) *1st year of life*: OR=1.27 (95% CI, 0.67-2.40) p=0.47 *2nd year of life*: OR=3.66 (95% CI, 1.36-9.87) p=0.01 *2 year lifetime:* OR=1.91 (95% CI, 1.09-3.37) p=0.03 Association with cord blood 25(OH)D (43.4 vs 3.7-17.4 nmol/L) *1st year of life:* OR=0.92 (95% CI, 0.45-1.85) p=0.81 *2nd year of life:* OR=4.65 (95% CI, 1.50-14.48) p=0.008 *2 year lifetime:* OR=1.70 (95% CI, 0.92-3.14) p=0.09	Maternal & cord blood 25(OH)D positively associated with children's risk for food allergy within first 2 years after birth.

Reference/Country	Population	Follow-up (y)	Mean baseline 25(OH)D (nmol/l)	Results	Comments
Type 1 diabetes					
Simpson *et al* (2011) US	Children at increased risk of type 1 diabetes (n=2644) Age 9m-10y.	8	Only reported in graph.	Risk of islet autoimmunity (IA) *(risk reported for SD difference in 25(OH)D – 20.3 nmol/L)*: HR=1.12 (95% CI, 0.88-1.43) p=0.36 25(OH)D ≤ 50 vs > 50: HR=0.68 (95% CI, 0.40-1.15) p=0.15 Risk of progression into diabetes in IA positive children (*risk reported for SD difference in 25(OH)D – 20.1 nmol/L)* HR=0.91 (95% CI, 0.68-1.22) p=0.54 25(OH)D ≤ 50 vs > 50: HR=0.44 (95% CI, 0.14-1.45) p=0.18	25(OH)D not associated with the risk of islet autoimmunity or progression to diabetes in IA positive children. Also no association between 25(OH)D & risk of IA in infants (n=128) aged 9 m
Sorensen *et al (*2012) Norway	Children who developed type 1 diabetes before age 15 y, born to women in study on Toxoplasma gondii infection during pregnancy (n=109 cases; 219 controls)	9	Cases – 65.8 (±26.5) Controls – 73.1 (27.2)	25(OH)D ≤54 nmol/L: OR=2.38 (95% CI, 1.12-5.07) 25(OH)D >54 nmol/L: OR+1.78 (95% CI, 0.85-3.74) 25(OH)D >69 ≤89 nmol/L: OR=1.35 (95% CI, 0.63-2.89) 25(OH)D >89 nmol/L: OR=1.0 (ref)	Maternal 25(OH)D <54 nmol/L associated with increased risk of type I diabetes risk in their child before the age 15 y.
Munger *et al* (2013) US	Active duty military personnel with US Department of Defense Serum Repository (n=923).	5.4	Cases (n=310) – 93.2 (42.1-172) Controls (n-613) – 97 (31-211)	Non-Hispanic whites (186 cases; 372 controls) 25(OH)D <75 nmol/L: RR=1 (ref) 25(OH)D 75-<100 nmol/L: RR=0.60 (95% CI, 0.38-0.97) 25(OH)D ≥ 100 nmol/L: RR=0.56 (95% CI, 0.35-0.90) p=0.03	Non Hispanic whites with 25(OH)D >100 nmol/L had lower risk of developing type I diabetes compared to those with 25(OH)D <75 nmol/. No association between 25(OH)D and type 1 diabetes in non-Hispanic blacks or Hispanics
Multiple sclerosis					
Salzer *et al* (2012) Sweden	Prospectively collected blood samples from MS cases & gestational samples from pregnant mothers whose offspring later developed MS (n=192 cases & 384 controls; n=37 gestational cases & 185 controls)	9	Cases – 40 (0-122) Controls – 39 (0-158) Gestational cases – 39 (19-103) Gestational controls – 40 (0-335)	25(OH)D ≥75 vs <75 nmol/L: OR=0.39 (95% CI, 0.16-0.98) Gestational 25(OH)D ≥75 vs <75 nmol/L: OR=1.8 (95% CI 0.53, 5.8)	In offspring, 25(OH)D ≥ 75 nmol/L associated with decreased risk of MS. No association between gestational 25(OH)D & MS risk in offspring.

Table 50: Meta-analyses/systematic reviews of RCTs & observational studies on vitamin D supplementation/25(OH)D concentration/genetic polymorphisms & infectious disease risk

Study	Methods	Results	Author conclusions
Tuberculosis (TB) – genetic studies			
Gao *et al* (2010)	Selection criteria: *Inclusion:* Case-control and cohort studies with original data on association between VDR polymorphisms and TB. Only polymorphisms FokI: rs10735810 C>T, TaqI: rs731236 T>C, ApaI: rs7975232 A>C, BsmI: rs1544410 A>G were considered. *Exclusion:* studies not in English or Chinese	23 studies: 13 in Asian populations, 8 in African populations, 2 in South American populations *Genotype FokI* (ff vs FF) Asian (12 studies): OR=2.0 (95% CI, 1.3-3.2) p<0.1 African (5 studies): OR=1.0 (95% CI, 0.7-1.3) p=0.8 S American (2 studies): OR=0.8 (95% CI, 0.4-2.0) p=0.6 All: OR 1.5 (95%CI 1.1, 2.0) p<0.1 *Genotype TaqI* (tt vs TT) Asian (10 studies): OR=1.4 (95% CI, 0.9-2.1) p=0.1 African (8 studies): OR=1.1 (95% CI, 0.6-2.1) p=0.7 S American (2 studies): OR=1.8 (95% CI, 0.5-6.4) p=0.4 All: OR=1.3 (95% CI, 0.9-1.9) p=0.2 *Genotype ApaI* (aa vs AA) Asian (6 studies): OR=1.3 (95% CI, 0.4-4.5) p=0.7 African (6 studies): OR=1.8 (95% CI, 1.2-2.8) p<0.1 All: OR=0.9 (95% CI, 0.7-1.2) p=0.4 *Genotype BsmI* (bb vs BB) Asian (6 studies): OR=0.5 (95% CI, 0.4-0.8) p<0.1 African (4 studies): OR=1.2 (95% CI, 0.8-1.6) p=0.4 All: OR=0.8 (95% CI, 0.6-1.3) p=0.2	Significant inverse association observed for *Bsm*I bb genotype & marginal significant associations *Taq*I & *Apa*I polymorphisms. However, no significant associations among Africans or South Americans.
Lewis *et al* (2005)	Selection criteria: *Inclusion:* Studies reporting *FokI* (rs10735810) and *TaqI* (rs731236) genotype frequencies in pulmonary TB (PTB) patients and controls	8 studies *FokI* - 6 studies (841 cases, 1419 controls) Ff vs FF: OR=1.12 (95% CI, 0.67-1.86) fF vs FF: OR=0.99 (95% CI, 0.81-1.22) *TaqI* - 8 studies (1614 cases, 1883 controls) tt vs TT: OR=1.00 (95% CI, 0.59-1.70) Tt vs TT OR=0.95 (95% CI, 0.80-1.14)	Results inconclusive as studies were underpowered .
Respiratory tract infections (RTI)			
Mao & Huang (2014)	Selection criteria: *Inclusion*: RCTs; healthy patients; exposure was vitamin D supplementation; outcome of interest was RTI; relative risk reported for vitamin D supplementation compared with placebo. *Exclusion*: low quality publications in terms of the modified Jadad score.	7 RCTs (n= 4827); age range 1-63y (1 RCT in children), vitamin D supplementation ranged from 7.5 to 170 µg/d ; duration, 1.75 -18 months. Vitamin D supplementation compared to control grp RR=0.98 (95% CI, 0.93-1.03) (p=0.45)	Vitamin D supplementation did not reduce risk of RTI.

Charan *et al* (2012)	Selection criteria: *Inclusion*: Randomised placebo controlled clinical trials. Only trials where results were given in categorical variables. *Exclusion:* Non randomised, clinical trials without controls and clinical trials showing results in continuous variables.	5 trials in children & adults (n not reported); supplementation dose ranged from 10-50µg/d OR=0.58 (95% CI, 0.417-0.812) p=0.001 (random model) OR=0.62 (95%CI, 0.488-0.776) p=0.000 (fixed model) Adults (*3 trials)* OR=0.54 (95% CI, 0.278-1.063) p=0.075 (random model) OR=0.65 (95% CI, 0.472-0.904) p=0.010 (fixed model) Children (*2 trials)* OR=0.58 (95% CI, 0.416-0.805) p=0.001 (random model) OR=0.58 (95% CI, 0.416-0.805) p=0.001 (fixed model)	RTIs significantly lower in vitamin D supplemented group compared to control group. After separating the studies in children from those of adults, the result only remained significant in children.
Bergman *et al* 2013	Selection criteria: *Inclusion:* Randomised trials, reporting incident RTI as primary or secondary outcome. Eligible outcomes included relative measures of infection risk or absolute numbers of patients experiencing at least one episode of RTI. Studies reporting composite endpoints deemed to reflect infectious episodes. Number of RTI episodes or days with RTI per patient were also considered *Exclusion:* Studies addressing TB or fungal infections	11 trials (n= 5660); mean age, 16 y; mean vitamin D dose, 40µg. OR=0.64 (95% CI, 0.49-0.84) p=0.001 Daily vitamin D: OR=0.51 (95% CI, 0.39-0.67) Large bolus (once/ month): OR=0.86 (95% CI, 0.62, 1.20)	Vitamin D supplementation significantly reduced risk of RTI. When studies separated by dosing interval, the protective effect was only significant in the studies that administered vitamin D daily.
Jolliffe *et al* (2013) Vitamin D in the prevention of acute respiratory infection: systematic review of clinical studies	Selection criteria: *Inclusion:* cross-sectional, case-control, cohort studies, clinical trials investigating relationship between serum concentration of vitamin D metabolites of clinical manifestations of vitamin D deficiency, or effect of dietary intake or administration of vitamin D or its analogues, on risk of acute respiratory infection or acute exacerbation of asthma or COPD. *Exclusion:* studies relating to TB.	39 studies (4 cross-sectional, 8 case-control, 13 cohort & 14 intervention studies). Meta-analysis not conducted. Out of 13 cohort studies, 7 reported associations between low 25(OH)D and susceptibility to ARI, 2 suggest that 1,25(OH)2D may be protective, 3 found no association, 1 reports a positive association between high maternal 25(OH)D in late pregnancy & increased risk of LRTI in offspring during infancy. Out of 14 clinical trials, 7 found vit D supplementation protected against ARI, 6 reported no effect & 1 reported adverse effects on pneumonia incidence	.

Table 51: RCTs on vitamin D supplementation and infectious disease risk

Study/ country	Population/ sample size	Intervention/study design	Baseline 25OHD (nmol/L) (SD)	Post intervention 25(OH)D (nmol/L)	Outcome	Results	Conclusion
Respiratory tract infection							
Dubnov-*Raz et al* (2015) Israel	Swimmers (n=55) with 25(OH)D < 75 nmol/L Mean age: 15 years	1. 50 µg/d vitamin D3 2. Placebo Duration: 12 wks	1. 60.7 (12.7) 2. 60.9 (11.7)	1. 73.9 (16.2) 2. 50.7 (10.2)	Frequency, duration and severity of URIs.	No between group differences in frequency, severity or duration of URIs.	Vitamin D3 supplementation does not reduce URI burden.
Goodall *et al* (2014) Canada	University students (n=600) Median age: 19 y	1. Vitamin D3 (250 µg/wk) + gargling (with water 3X/d) 2. Placebo + gargling (with water 3X/d) 3. Vitamin D3 (250 µg/wk) 4. Placebo Duration8 wks.	Not reported	Not reported	Incidence of clinical URTI; defined as participant's perception of "cold" plus ≥ symptoms (runny/stuffy nose, congestion, cough, sneezing, sore throat, muscle aches, or fever).	Vit D compared to placebo: *Reported by students* RR=0.79 (95% CI, 0.61-1.03; p=0.09). *laboratory confirmed URTI* RR=0.54 (95% CI: 0.34-0.84; p=0.007)	No significant difference between groups for incidence of symptomatic clinical URTI. Vitamin D3 treatment associated with significantly lower risk of laboratory confirmed URTI.
Grant *et al* (2015) New Zealand	Pregnant women (n=260) Mean age (y): 1. 28 2. 27 3. 26	Women & infant pairs assigned to 1 of 3 groups: 1. placebo & placebo 2. 25µg/d & 10 µg/d vit D 3. 50µg/d & 20µg/d vit D. Duration: Women: 27 wks to birth Infants: birth to 18 m	<u>Women</u> 1. 55 2. 57 3. 55	<u>36 wks gestation</u> Women 1. 50 2. 97 3. 102 Infant (age 6m) 1. 77 2. 85 3. 95	Number of primary care visits for ARI.	Compared with placebo group (99%), proportion of children making any ARI visits was smaller in high dose group (87%, p=0.004) but not lower dose vitamin D group (95%; p=0.17).	Vitamin D3 supplementation during pregnancy reduced primary care visits for ARI during early childhood in high dose vitamin D group.
Martineau *et al* (2015) UK	Adults in sheltered housing accommodation blocks (including carers) (n=240) Mean age: 67y	<u>Intervention</u> 1. *Residents :*2,400 µg D3/2 monthly + 10 µg/d 2. *Carers:* 3,000 µg D3/2 monthly. <u>Control</u> 1. *Residents:* placebo/2 monthly + 10 µg vit D3/d 2. *Carers:* placebo/2 monthly Duration: 12 months.	<u>Intervention</u> 42.4 (23.4) <u>Control</u> 43.6 (22.6)	<u>Intervention</u> 65.5 (19.8) (2 m) & 85.3 (24.3)(12 m) <u>Control</u> 52.9 (21.7) (2 m) & 59.1 (26.0)(12 m)	Time to first ARI	Allocation to intervention vs control arm: <u>ARI</u> HR: 1.18 (95% CI, 0.80 –1.74; p=0.42) <u>URI</u> HR: 1.48 (95% CI, 1.02-2.16; p=0.039)	Addition of intermittent bolus-dose vitamin D3 to a daily low-dose regimen did not influence risk of ARI in older adults & their carers but was associated with increased risk & duration of URI.

Study/ country	Population/ sample size	Intervention/study design	Baseline 25OHD (nmol/L) (SD)	Post intervention 25(OH)D (nmol/L)	Outcome	Results	Conclusion
Rees *et al* (2013) USA	Participants from larger double blind multi centre vitamin D trial on prevention of large bowel adenomas (n=759) Age 45-75 years	1. D3 (25µg/d) + Ca (1200 mg/d) 2. Placebo Duration: 3y	At enrolment 1. 61.9 2. 63.1 At start of study 1. 83.1 2. 62.6	Not reported	Number of episodes (winter) URTI 1. 275 (71.1%) 2. 252 (71.8%) Colds 1. 267 (69%) 2. 239 (68.1%) Influenza like illness 1. 46 (11.9%) 2. 51 (14.5%)	URTI RR=0.93 (5% CI, 0.79-1.09) Colds RR=0.93 (95% CI, 0.78-1.10) Influenza like illness RR=0.95 (95% CI, 0.62-1.46)	Vitamin D supplementation (25µg/d) did not significantly reduce incidence or duration of upper RTI in adults with a baseline serum 25 (OH)D >30 nmol/L.
Simpson *et al* (2015) Australia	Adults (n=34) Mean age: 1. 30y 2. 35y	1. 500 µg D3/week 2. Placebo Duration: 17 wks	1. 60.5 2. 76.4 (p=0.04)	1. 100.7 (23.9) 2. 56 (24.2)	Frequency, duration and severity of acute infections.	<u>Infection risk</u> *All participants* HR: 0.83 (95% CI, 0.53-1.31) *Participants with baseline 25(OH)D < 40 nmol/L* (n=4) HR: 0.56 (95% CI, 0.32-0.96; p=0.007) *Clinically verified infections* HR: 0.27 (95% CI, 0.07-1.00; p=0.05)	No difference between groups for infection risk, duration or severity. A treatment effect was observed amongst those with 24(OH)D < 40 nmol/L at start of study. However, this only related to 4 participants.
Urashima *et al* (2014) Japan	Schoolchildren (n=247) Age: 15-18 y	1. 50 µg D3/d 2. Placebo 2 months	Not reported	Not reported	Incidence of influenza A (diagnosed by a RIDT by medical doctors).	<u>Total 2 month period</u> RR=1.11 (95% CI, 0.57-2.18; p=0.75) <u>After first month</u> RR=0.17 (95% CI, 0.04-0.77; p=0.009)	Vitamin D supplementation did not decrease the overall incidence of RIDT-positive influenza A.

Table 52: Observational studies on 25(OH)D concentration and infectious disease risk

Study/Country	Population	Follow-up)	Mean baseline 25(OH)D (nmol/L)	Results	Authors' Conclusions
Tuberculosis (TB)					
Arnedo-Pena *et al* (2011) Spain	Contacts of TB patients (n=202) Mean age: 39.9 y (Spanish); 32.0 y (19.9) (non-Spanish) (p> 0.05)	13 m	Cases: 43.7 (14.0) Controls: 64.7(34.2) (p=0.041)	Cases with positive TST conversion (n=11) Controls with negative TST results (n=82) Association between 25(OH) Dand TST conversion ≥ 75 vs <50 nmol/L OR=0.10 (95% CI, 0.00-0.76; p=0.019).	25(OH) D ≥ 75 nmol/L associated with significantly reduced risk of TST conversion.
Arnedo-Pena *et al* (2015a) Spain	Contacts of TB patients (n=572) Mean age: Cases – 42.3 (±24)/non-cases – 38 (±14)	1.6 y(±0.9)	Cases: 34 (5.7) Controls: 64 (31.7)	3 new cases occurred Association between 25(OHD and TB incidence HR=0.89 (95% CI, 0.88-0.99); p=0.034	25(OH)D concentration associated with TB incidence.
Arnedo-Pena et al (2015b) Spain	Contacts of pulmonary TB patients (n=247)	8-10 wks	Cases: 67.9 (28.5) Controls: 51.7 (29.7) p=0.028	198 contacts screened twice (49 lost in follow up) 18 TBIC cases. Per 2.5 nmol/L increase 25(OH)D; RR=0.94 (95% CI, 0.90-0.99, p=0.015)	25(OH)D concentration associated with TB incidence.
Talat *et al* (2010) Pakistan	Contacts of TB patients (n=109)	4 y	Median (IQR) Cases (n=20) 19.7 (11.7-25.7) Controls (n=100): 24.0 (14.5-47.7)	8 new cases occurred Per relative 1-log decrement in 25(OH)D (adjusted for age & sex): RR =5.1 (95% CI, 1.2-21.3; p=0.03)	TB progression significantly associated with lower 25(OH)D concentrations
Respiratory tract infections (RTI)					
Shin *et al* (2013) Korea	Newborns from birth cohort (n=525)	6 m	Median (IQR) 32 (21.4-53.2)	Cord 25(OH)D ≥75 vs <25 nmol/L *RTI:* OR=3.56 (95% CI, 1.52-8.34) ptrend=0.0015 *Acute nasopharyngitis:* OR=5.21 (95% CI, 1.91-14.27) ptrend=0.0004 *Otitis media:* OR=2.83 (95% CI, 0.33-23.91) ptrend=0.3745 *Bronchiolitis:* OR=2.65 (95% CI, 0.32-22.20) ptrend=0.4485	Cord blood 25(OH)D inversely associated with risk of acute nasopharyngitis.
Magnus *et al* (2013) Norway	Children (n=1248) from mother & child cohort Age: 36 m	36 m	Maternal (median 18 wks gestation): 73.7 (±23.7)	Associations of 20 nmol/L increase in maternal 25(OH)D (18 wks) with LRTI frequency 1-2 vs 0 LRTIs: RR=0.98 (95% CI, 0.87-1.12) ≥ 3 vs 0 LRTIs: RR=0.74 (95% CI, 0.58-0.93)	Higher maternal mid-term pregnancy 25(OH)D associated with reduced risk of recurrent LRTIs at 36m.

Science *et al* (2013) Canada	Children & adolescents in RCT on effect of influenza vaccination on viral infection rates (n=947) Age:3-15y	6 m	Median (IQR): 62.0 (51.0–74.0)	25(OH)D <50 vs ≥ 50 nmol/L: HR=1.67 (95% CI, 1.16–2.40) p=0.006 25(OH)D <75 vs ≥ 75 nmol/L: HR=1.51 (95% CI, 1.10–2.07) p=0.011	Lower 25(OH)D associated with increased upper RTI risk.
Pneumonia					
Jovanovich *et al* (2014) USA	Individuals hospitalised with pneumonia; 25(OH)D measured 3–15 m prior to admission (n=66 cases/66 controls) Mean age: 60y (±17)	15 m	Median (IQR) Cases: 70.1 (62.2-79.6) Controls: 79.3 (71.1-88.1)	25(OH)D: <75 vs ≥75 nmol/L: OR=1.03 (95% CI,0.51-2.09); p=0.93 <50 vs ≥50 nmol/L: OR=0.96 (95% CI, 0.35-2.61); p=0.96 <37 vs ≥37 nmol/L: OR=0.57 (95% CI, 1.08-5.08); p=0.03	Increased risk of hospitalisation for pneumonia with 25(OH)D <37 nmol/L.
Aregbesola *et al* (2013) Finland	Participants in Kuopio Ischemic Heart Disease Risk Factor study (n=1421) Mean age: 62.5 (±6.5) y	9.8 y	43.5 (±17.8)	25(OH)D <34 vs ≥ 51 nmol/L: RR=2.6 (95% CI, 1.4-5.0) 25(OH)D 34-51 vs ≥ 51 nmol/L: RR=1.5 (95% CI, 0.7-2.9) ptrend=0.005	Inverse association between 25(OH)D & risk of pneumonia.

Neuropsychological functioning

Table 53: Systematic reviews/meta-analyses of RCTs & observational studies on vitamin D supplementation/25(OH)D concentration and neuropsychological functioning

Study	Methods	Results	Authors' conclusions
Cognition & dementia			
van der Schaft *et al* (2013)	Selection criteria: *Inclusion*: observational studies where vitamin D defined as concentration in serum or as dietary intake, cognition defined as score on a cognitive function test, study participants were adults, measure of association available. *Exclusion:* animal studies, studies in children, study about vitamin D supplementation and no focus on association, expert opinion.	25 cross-sectional studies (n=48,680), age, 20-80 y 6 prospective studies (n=10,896), age, 65 y+, mean follow up, 4-7 y Meta-analysis could not be performed due to large variability in measures used to assess vitamin D exposure, cognitive function. *Cross-sectional studies:* statistically significant worse outcome on ≥ 1 cognitive function tests or a higher frequency of dementia with lower 25(OH)D or vitamin D intake in 72% of studies. *Prospective studies*: Statistically significant decline on ≥ 1 cognitive function tests or higher frequency of dementia in participants with lower 25(OH)D or vitamin D intake in 67% of studies.	Hypovitaminosis D associated with worse outcome on 1 or more cognitive function tests or a higher frequency of dementia in cross-sectional & prospective studies
Depression			
Anglin *et al* (2013)	Selection criteria: *Inclusion:* RCTs, case-control, cross-sectional & cohort studies, adults 18 y +. Depression reported as outcome of interest and vitamin D measurements as a risk factor or intervention. *Exclusion:* Not reported.	1 case-control study, 3 cohort studies, 10 cross-sectional studies (n=31,424) 9 cross-sectional studies: Lowest vs highest 25(OH)D OR=1.31 (95% CI, 1.0-1.71) p=0.05 3 cohort studies: Lowest vs highest 25(OH)D HR 2.21 (95%CI 1.40, 3.49) p=0.0007	Low 25(OH)D concentration associated with depression.
Spedding *et al* (2014)	Selection criteria: *Inclusion:* RCTs with vitamin D supplementation *Exclusion:* trials that were not RCTs or used surrogate interventions	15 RCTs identified; only 2 included in meta-analysis because same depression measure used. SMD=0.78 (95% CI, 0.24-1.27)	Vitamin D has therapeutic effects in depression.
Li *et al* (2015)	Selection criteria: *Inclusion:* RCTs with primary comparison being oral vitamin D versus placebo; adults at risk of depression, having depression symptoms or having a primary diagnosis of depression. *Exclusion:* Not reported.	6 RCTs (n=1,203); 5 studies included adults at risk of depression and 1 included adults diagnosed with depression. SMD = -0.14 (95% CI, 0.41 – 0.13, p=0.32); OR = 0.93 (95% CI, 0.54-1.59; p=0.79) Substantial heterogeneity (I^2=77%; p<0.01)	Vitamin D supplementation has no effect on depression.
Schizophrenia			
Valipour *et al* (2014)	Selection criteria: *Inclusion:* observational studies with measures of serum 25(OH)D concentration in schizophrenic patients. *Exclusion:* Studies which did not report serum 25(OH)D concentrations.	19 studies (n=2,804): 8 cross-sectional, 10 case-control, 1 nested case-control study; age, 18-65 y Mean difference in 25(OH)D between schizophrenic patients & controls: 15 nmol/L (95% CI, -27 to -3) Odds of schizophrenia 25(OH)D < 50 vs >50 nmol/L: OR=2.16 (95% CI, 1.32-3.56)	Participants with 25(OH)D <50 nmol/L more likely to have schizophrenia.

Table 54: RCTs of vitamin D supplementation and risk of cognition & dementia

Study/ country	Study population	Intervention	Mean baseline 25OHD (nmol/L)	Post intervention 25(OH)D (nmol/L)	Results	Authors' conclusions
Przybelski *et al* (2008) US	Nursing home residents with 25(OH)D ≤ 62.4 nmol/L (n=63) Mean age: Intervention gp: 86.2(±2.3) y Control gp: 87.4 (±0.9) y	1. D2 (1250 µg 3 x/wk) 2. No placebo or treatment Duration: 4 wks Unblinded study	1. 43.2 (3) 2. 86.9 (4.5)	1. 159 (8.5) 2. unchanged	No difference between groups in results for timed walk test, animal fluency, clock drawing & neuropsychiatric inventory.	Findings do not support role for vitamin D in normal cognition and mood.
Stein *et al* (2011) Australia	Community dwelling individuals with mild/moderate Alzheimer's disease(AD) (n=32) Median age (IQR) = 77.5 (69-80) y	All: D2 (25µg/d) throughout trial After 8 wks: 1. D2 (300 µg 3x/d, then 0-2x/d to maintain 25(OH)D at 130-175 nmol/L in wks 2, 4 & 6) 2. Placebo Duration: 16 weeks	Median (IQR): 49 (39-67)	After run-in 8 wks, median (IQR): 1. 60 (56-70) 2. 64 (48-72) After further 8 wks, median (IQR) 1. 187 (160-240) 2. 72 (63-81)	No change in AD assessment-scale cognitive subscale nor Disability Assessment in Dementia	High dose vitamin D provides no benefit for cognition or disability.
Rossom *et al* (2012) USA	Participants in Women's Health Initiative (WHI) calcium & vitamin D trial & the WHI Memory study (n=4143 women) Mean age:71y	1. Vitamin D3 (10 µg/d) & calcium carbonate (1000 mg/d) 2. Placebo Duration: 7.8 y	1.50 (n=150) 2.48 (n=143)	Not reported.	Vit D/Ca group vs placebo HR=0.94 (95% CI, 0.72-1.24) p=0.72	Incidence of cognitive impairment did not differ between groups.

Abbreviations used in studies

25(OH)D	25-hydroxyvitamin D
ACE inhibitor	Angiotensin-converting-enzyme inhibitor
AFB	Acid-fast bacilli
ALP	Alkaline phosphatase
ARI	Acute respiratory infection
BMC	Bone mineral content
BMD	Bone mineral density
BP	Blood pressure
Ca	Calcium
CC	Case-control study
CI	Confidence interval
DBP	Diastolic blood pressure
HR	Hazard ratio
IQR	Inter-quartile range
IRR	Incident rate ratio
LTBI	Latent tuberculosis infection
MD	Mean difference
NCC	Nested case control study
OR	Odds ratio
P	Phophorus
PTH	Parathyroid hormone
RaR	Rate ratio
RCT	Randomised controlled trial
RITD	Rapid influenza diagnostic test
RR	Relative risk
RTI	Respiratory tract infection
SBP	Systolic blood pressure
SD	Standard deviation
SMD	Standardised mean difference
SPPB	Short physical performance battery
TB	Tuberculosis
TBIC	Tuberculosis infection conversion
URI	Upper respiratory infection
URTI	Upper respiratory tract infection
VDDR	Vitamin D deficiency rickets

ANNEX (3)

Dietary vitamin D intakes and serum/plasma 25(OH)D concentrations in the UK

Vitamin D intakes in the UK

Table 1: Percentage contribution of food groups (food sources) to daily vitamin D intake (µg) for non-breastfed children aged 4-18 months

UK Diet and Nutrition Survey of Infants and Young Children aged 4-18 months (2011)				
Food group[a]	Age group (months)			
	4-6	7-9	10-11	12-18
Non-infant specific foods:				
Cereals and cereal products	0	0	1	7
Milk and milk products	1	3	6	16
Eggs and egg dishes	0	0	2	7
Fat spreads[b]	0	1	3	11
Meat and meat products and dishes, total	0	1	3	13
Fish and fish dishes	0	1	2	5
Vegetables, potatoes	0	0	0	1
Savoury snacks	0	0	0	0
Fruit	0	0	0	0
Sugar, preserves and confectionery	0	0	0	0
Beverages	0	0	0	0
Miscellaneous	0	0	0	1
Infant specific foods:				
Infant formula	85	80	72	29
of which:				
'First milk'	*35*	*19*	*14*	*1*
'Hungrier babies milk'	*22*	*8*	*4*	*1*
Follow-on milk	*27*	*48*	*49*	*11*
'Growing up milk'	*0*	*0*	*3*	*14*
Soy milk	*0*	*1*	*0*	*0*
Other milk products[c]	*1*	*5*	*2*	*2*
Commercial infant foods:	12	12	10	9
of which:				
Meat and fish based products and dishes	*2*	*2*	*2*	*2*
Cereal based foods and dishes	*7*	*7*	*6*	*5*
Snacks (sweet and savoury)	*2*	*2*	*2*	*2*
Commercial infant beverages	0	0	0	0
Average daily Vitamin D intake (food sources) µg[d]	9.8	8.7	7.5	3.5
Bases (unweighted)	*240*	*489*	*381*	*1177*

a Some food groups are not included due to small numbers of consumers; e.g. nuts and seeds and savoury snacks.
b Some oils which are used as a condiment on bread and salads are included in this food group; however this food groups does not include cooking oils.
c Includes hypoallergenic, goats and "goodnight" milk.
d Vitamin D intake does not include values for breastfed children as the vitamin D content of breast milk is not known.

Table 2: Percentage contribution of food groups to average daily vitamin D intake (µg), by age (*1.5 years and over)*

UK National Diet and Nutrition Survey: year 1, 2, 3 and 4 combined (2008/09 - 2011/12)					
Food group[a]	**Age group (years)**				
	1.5-3	4-10	11-18	19-64	65+
Cereals and cereal products *of which*	14	20	17	13	13
Pasta, rice, pizza and other miscellaneous cereals	2	2	4	2	1
High fibre breakfast cereals	2	2	2	2	3
Other breakfast cereals	6	7	6	4	3
Biscuits	0	1	1	0	0
Buns, cakes, pastries and fruit pies	3	6	4	3	4
Puddings	1	2	2	1	2
Milk and milk products *of which*	24	13	6	5	6
Other milk and cream	7	1	0	0	1
Cheese	4	3	2	2	2
Yoghurt, fromage frais and other dairy desserts	11	6	2	1	2
Ice cream	2	3	2	1	1
Eggs and egg dishes	9	8	9	13	13
Fat spreads[b] *of which*	20	21	20	19	19
Butter	1	1	1	1	2
Reduced fat spread polyunsaturated (41-75% fat)	4	4	3	4	4
Reduced fat spread not polyunsaturated (41-75% fat)	12	12	13	11	8
Low fat spread polyunsaturated (18-39% fat)	2	3	2	3	3
Low fat spread not polyunsaturated (18-39% fat)	0	1	1	1	1
Meat and meat products *of which*	21	25	35	30	23
Bacon and ham	2	3	4	4	4
Beef, veal and dishes	3	4	5	6	5
Lamb and dishes	1	1	2	2	2
Pork and dishes	1	1	2	3	2
Coated chicken and turkey	1	2	3	1	0
Chicken, turkey and dishes	1	3	6	5	3
Liver and dishes	0	0	0	0	1
Burgers and kebabs	1	2	3	2	0

Table 2 (continued)

UK National Diet and Nutrition Survey: year 1, 2, 3 and 4 combined (2008/09 - 2011/12)					
Food group[a]	**Age group (years)**				
	1.5-3	**4-10**	**11-18**	**19-64**	**65+**
Meat and meat products *contd.*					
Sausages	7	7	6	5	3
Meat pies and pastries	2	2	3	2	2
Other meat, meat products and dishes	1	1	1	1	1
Fish and fish dishes *of which*	8	8	9	17	23
Other white fish, shellfish, fish dishes and canned tuna	4	3	5	5	2
Oily fish	4	5	4	11	20
Vegetables and potatoes *of which*	1	1	2	2	1
Vegetables (not raw) including vegetable dishes	0	0	1	1	0
Other potatoes, potato salads and dishes	1	1	1	1	1
Savoury snacks	0	0	0	0	0
Nuts and seeds	0	0	0	0	0
Fruit	0	0	0	0	0
Non-alcoholic beverages[c]	0	0	0	0	0
Alcoholic beverages	0	0	0	0	0
Miscellaneous[d] *of which*	3	1	1	2	3
Dry weight beverages	1	1	0	0	2
Savoury sauces, pickles, gravies and condiments	0	0	1	1	0
Commercial toddler foods	1	0	0	0	0
Bases (unweighted)	*604*	*1277*	*1497*	*2697*	*753*

a Food groups that contribute <0.5% to intake across all age/sex groups are excluded from the table. All other food groups are included.
b Some oils which are used as a condiment on bread or salads are included in this food group; however this food group does not include oils or fats used in cooking.
c Non-alcoholic beverages are reported as consumed with diluent water.
d In addition to dry weight beverages; soup, manufactured/retail and homemade; savoury sauces, pickles, gravies and condiments; and commercial toddler foods, Miscellaneous also includes nutrition powders and drinks.

Table 3: Percentage contribution of food groups to average daily vitamin D intake – low income/materially deprived consumers (2y and above)

Low Income Diet and Nutrition Survey 2003/05												
Food group	***Age group** (years)*											
	Boys		***Men***				***Girls***		***Women***			
	2-10	*11-18*	*19-34*	*35-49*	*50-64*	*65+*	*2-10*	*11-18*	*19-34*	*35-49*	*50-64*	*65+*
Cereals and cereal products	*21*	*16*	*9*	*7*	*9*	*14*	*20*	*14*	*13*	*14*	*13*	*19*
of which:												
Breakfast cereals	*6*	*3*	*3*	*1*	*4*	*3*	*6*	*4*	*5*	*5*	*5*	*6*
Buns, cakes, pastries and fruit pies	*8*	*6*	*3*	*3*	*3*	*6*	*8*	*5*	*4*	*4*	*4*	*9*
Puddings	*3*	*2*	*1*	*0*	*1*	*1*	*1*	*1*	*1*	*2*	*1*	*2*
Milk and milk products	*5*	*4*	*3*	*3*	*5*	*4*	*7*	*4*	*3*	*4*	*4*	*4*
Eggs and egg dishes	*6*	*8*	*15*	*13*	*15*	*11*	*7*	*8*	*10*	*9*	*11*	*9*
Fat spreads	*26*	*29*	*24*	*32*	*27*	*28*	*25*	*26*	*26*	*25*	*27*	*25*
of which:												
Butter	*1*	*1*	*1*	*3*	*1*	*2*	*1*	*2*	*1*	*2*	*2*	*3*
Margarine and other fats and oils	*4*	*5*	*3*	*3*	*2*	*1*	*3*	*4*	*4*	*2*	*1*	*1*
Reduced fat spread (41-75% fat)	*17*	*18*	*15*	*19*	*18*	*17*	*15*	*15*	*15*	*15*	*14*	*14*
Low fat spread (18-39% fat)	*5*	*4*	*4*	*8*	*5*	*8*	*5*	*5*	*5*	*4*	*10*	*7*
Meat and meat products	*30*	*36*	*37*	*35*	*32*	*27*	*28*	*37*	*31*	*34*	*27*	*24*
of which:												
Bacon and ham	*2*	*3*	*3*	*4*	*4*	*4*	*3*	*3*	*3*	*4*	*4*	*4*
Beef, veal and dishes	*9*	*8*	*10*	*12*	*12*	*11*	*7*	*10*	*11*	*11*	*9*	*10*
Coated chicken and turkey	*3*	*3*	*2*	*1*	*1*	*0*	*4*	*4*	*2*	*1*	*1*	*0*
Chicken, turkey and dishes	*3*	*5*	*7*	*5*	*3*	*3*	*3*	*5*	*5*	*5*	*4*	*3*

Table 3 (continued)

Food group	***Age group** (years)*											
	Boys		***Men***				***Girls***		***Women***			
	2-10	*11-18*	*19-34*	*35-49*	*50-64*	*65+*	*2-10*	*11-18*	*19-34*	*35-49*	*50-64*	*65+*
Liver and liver dishes	*0*	*0*	*0*	*0*	*2*	*1*	*0*	*0*	*0*	*0*	*0*	*1*
Burgers and kebabs	*3*	*3*	*4*	*2*	*1*	*0*	*1*	*3*	*2*	*3*	*1*	*0*
Sausages	*6*	*6*	*6*	*5*	*4*	*4*	*5*	*6*	*5*	*3*	*2*	*2*
Meat pies and pastries	*4*	*6*	*3*	*4*	*2*	*3*	*4*	*5*	*3*	*4*	*4*	*2*
Other meat, meat products & dishes	*1*	*1*	*1*	*1*	*3*	*2*	*2*	*1*	*1*	*2*	*1*	*2*
Fish and fish dishes	*4*	*5*	*10*	*6*	*10*	*12*	*6*	*7*	*12*	*10*	*14*	*13*
of which:												
Canned tuna and dishes	*3*	*4*	*7*	*3*	*1*	*1*	*3*	*5*	*5*	*4*	*3*	*1*
Oily fish	*1*	*1*	*3*	*2*	*8*	*10*	*2*	*2*	*6*	*6*	*11*	*11*
Vegetables	*0*	*0*	*0*	*1*	*0*	*0*	*1*	*1*	*1*	*1*	*1*	*1*
Potatoes and savoury snacks	*2*	*2*	*1*	*1*	*1*	*1*	*2*	*2*	*2*	*2*	*1*	*1*
Fruit and nuts	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Sugar, preserves and confectionery	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Beverages[a]	*4*	*0*	*0*	*1*	*0*	*0*	*3*	*0*	*0*	*1*	*0*	*0*
Miscellaneous[b]	*0*	*0*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*2*	*3*
Base (unweighted)	*239*	*200*	*194*	*226*	*258*	*268*	*278*	*215*	*483*	*494*	*336*	*537*

[a] Includes soft drinks, alcoholic drinks, tea, coffee and water.

[b] Includes powdered beverages (except tea and coffee),soups, sauces, condiments and artificial sweeteners.

Table 4: Percentage contribution of food groups to average daily vitamin D intake (µg) in Scotland, by age (1.5y and above)

National Diet and Nutrition Survey: year 1, 2, 3 and 4 combined (2008/09 - 2011/12) Scotland					
Food group[a]	**Age group** (years)				
	1.5-3	4-10	11-18	19-64	65+
Cereals and cereal products *of which:*	15	19	17	16	16
Pasta, rice, pizza and other miscellaneous cereals	2	3	3	3	1
High fibre breakfast cereals	2	2	2	3	2
Other breakfast cereals	6	7	5	6	5
Biscuits	0	1	1	0	1
Buns, cakes, pastries and fruit pies	4	6	4	3	5
Puddings	0	1	1	1	1
Milk and milk products *of which:*	25	13	6	4	4
Other milk and cream	6	1	0	0	0
Cheese	4	2	2	2	1
Yoghurt, fromage frais and other dairy desserts	13	7	1	1	1
Ice cream	1	4	2	1	1
Eggs and egg dishes	7	7	8	11	16
Fat spreads *of which:*	20	21	21	18	19
Butter	1	1	1	1	2
Reduced fat spread polyunsaturated (41-75% fat)	3	5	4	3	4
Reduced fat spread not polyunsaturated (41-75% fat)	11	13	12	9	8
Low fat spread polyunsaturated (18-39% fat)	4	3	3	3	4
Low fat spread not polyunsaturated (18-39% fat)	1	1	0	1	1
Meat and meat products *of which:*	22	27	36	32	24
Bacon and ham	2	4	4	5	4
Beef, veal and dishes	3	5	6	8	6
Lamb and dishes	0	0	1	1	2
Pork and dishes	0	1	2	2	1
Coated chicken and turkey	2	2	3	1	0
Chicken, turkey and dishes	2	2	6	5	3
Burgers and kebabs	1	2	2	2	1
Sausages	7	8	8	6	3
Meat pies and pastries	3	3	3	2	3
Other meat, meat products and dishes	1	1	1	1	2

Table 4 (continued)

Food group[a]	Age group (years)				
	1.5-3	4-10	11-18	19-64	65+
Fish and fish dishes *of which:*	7	9	9	14	18
Other white fish, shellfish, fish dishes and canned tuna	4	5	4	5	2
Oily fish	3	3	4	10	15
Vegetables and potatoes *of which*:	1	1	1	2	1
Vegetables (not raw) including vegetable dishes	1	0	0	1	0
Other potatoes, potato salads and dishes	1	1	1	1	1
Savoury snacks	0	0	0	0	0
Nuts and seeds	0	0	0	0	0
Fruit	0	0	0	0	0
Sugar, preserves and confectionery	0	0	0	0	0
of which: Chocolate confectionery	0	0	0	0	0
Non-alcoholic beverages[c]	0	0	0	0	0
Alcoholic beverages	0	0	0	0	0
Miscellaneous[d]	3	1	1	2	2
Dry weight beverages	1	0	0	0	0
Soup, manufactured/retail and homemade	0	1	0	0	0
Savoury sauces, pickles, gravies and condiments	0	0	1	1	1
Commercial toddler foods	2	0	0	0	0
Average daily total vitamin D intake (µg)	1.8	2.0	2.1	2.7	3.2
Bases (unweighted)	*125*	*307*	*396*	*650*	*217*

[a] Food groups that contribute <0.5% to intake across all age/sex groups are excluded from the table. All other food groups are included.

[b] Some oils which are used as a condiment on bread or salads are included in this food group; however this food group does not include oils or fats used in cooking.

[c] Non-alcoholic beverages are reported as consumed with diluent water.

[d] In addition to dry weight beverages; soup, manufactured/retail and homemade; savoury sauces, pickles, gravies and condiments; and commercial toddler foods, Miscellaneous also includes nutrition powders and drinks.

Table 5: Percentage contribution of food groups to average daily vitamin D (µg) in Northern Ireland, by age (1.5y & above)

National Diet and Nutrition Survey: year 1, 2, 3 and 4 combined (2008/09 - 2011/12) Northern Ireland					
Food group[a]	**Age group** (years)				
	1.5-3	4-10	11-18	19-64	65+
Cereals and cereal products *of which:*	12	18	15	14	13
Pasta, rice, pizza and other miscellaneous cereals	2	1	2	1	0
High fibre breakfast cereals	1	3	2	3	2
Other breakfast cereals	5	7	6	5	3
Biscuits	0	1	1	0	0
Buns, cakes, pastries and fruit pies	4	6	4	3	7
Puddings	0	1	1	1	1
Milk and milk products *of which:*	17	12	5	3	4
Other milk and cream	2	0	0	0	0
Cheese	3	2	1	2	1
Yoghurt, fromage frais and other dairy desserts	11	6	1	1	2
Ice cream	1	4	2	1	1
Eggs and egg dishes	7	6	7	13	13
Fat spreads[c] *of which:*	28	26	24	22	27
Butter	2	2	2	3	4
Margarine and other fats and oils	0	1	0	1	2
Reduced fat spread polyunsaturated (41-75% fat)	7	3	4	2	2
Reduced fat spread not polyunsaturated (41-75% fat)	13	12	10	10	8
Low fat spread polyunsaturated (18-39% fat)	5	7	7	5	9
Low fat spread not polyunsaturated (18-39% fat)	0	1	0	1	3

Table 5 (continued)

National Diet and Nutrition Survey: year 1, 2, 3 and 4 combined (2008/09 - 2011/12) Northern Ireland					
Food group[a]	**Age group** (years)				
	1.5-3	4-10	11-18	19-64	65+
Meat and meat products *of which*:	26	31	41	35	22
Bacon and ham	3	5	6	5	3
Beef, veal and dishes	3	5	7	8	7
Lamb and dishes	0	1	1	1	1
Pork and dishes	1	1	2	3	3
Coated chicken and turkey	2	2	3	2	0
Chicken, turkey and dishes	1	4	6	5	3
Burgers and kebabs	2	2	3	3	0
Sausages	12	8	9	7	3
Meat pies and pastries	3	2	4	2	1
Other meat, meat products and dishes	0	0	0	1	1
Fish and fish dishes *of which*:	5	3	5	10	19
Other white fish, shellfish, fish dishes and canned tuna	1	2	3	4	4
Oily fish	4	1	1	6	15
Vegetables and potatoes *of which:*	1	2	1	1	1
Vegetables (not raw) including vegetable dishes	0	0	0	1	1
Other potatoes, potato salads and dishes	1	1	1	1	0
Savoury snacks	0	0	0	0	
Nuts and seeds	0	0	0	0	
Fruit	0	0	0	0	
Sugar, preserves and confectionery *of which:*	0	1	0	0	0
Chocolate confectionery	0	1	0	0	0

Table 5 (continued)

National Diet and Nutrition Survey: year 1, 2, 3 and 4 combined (2008/09 - 2011/12) Northern Ireland					
Food group[a]	**Age group** (years)				
	1.5-3	4-10	11-18	19-64	65+
Non-alcoholic beverages[d]	0	0	0	0	0
Alcoholic beverages	0	0	0	0	0
Miscellaneous[e]	3	1	2	1	1
Savoury sauces, pickles, gravies and condiments	0	0	1	1	0
Commercial toddler foods	2	0	0	0	0
Average daily total vitamin D (µg)	1.8	1.9	2.1	2.6	3.6
Bases (unweighted)	*94*	*182*	*236*	*391*	*79*

a Food groups that contribute <0.5% to intake across all age/sex groups are excluded from the table. All other food groups are included.

b Due to small cell sizes, participants aged 65 years and over have only been reported as males and females combined.

c Some oils which are used as a condiment on bread or salads are included in this food group; however this food group does not include oils or fats used in cooking.

d Non-alcoholic beverages are reported as consumed with diluent water.

e In addition to dry weight beverages; soup, manufactured/retail and homemade; savoury sauces, pickles, gravies and condiments; and commercial toddler foods. Miscellaneous also includes nutrition powders and drinks.

Table 6: Percentage contribution of food groups to average daily vitamin D intake (µg) in Wales, by sex and age (*1.5 years and over)*

National Diet and Nutrition Survey: year 2, 3, 4 and 5 combined (2009/10 - 2012/13) Wales					
Food group[a]	**Age group** (years)				
	1.5-3	4-10	11-18	19-64	65+
Cereals and cereal products *of which:*	13	20	20	12	11
Pasta, rice, pizza and other miscellaneous cereals	2	2	3	2	1
High fibre breakfast cereals	2	2	3	2	2
Other breakfast cereals	6	9	8	5	2
Biscuits	0	0	0	0	0
Buns, cakes, pastries and fruit pies	3	5	4	2	5
Puddings	0	2	1	1	1
Milk and milk products	24	11	4	5	5
Other milk and cream	5	0	0	1	2
Cheese	2	2	1	2	1
Cheddar cheese	0	1	1	1	1
Yoghurt, fromage frais, and other dairy desserts	13	7	1	1	1
Ice cream	3	2	2	1	1
Eggs and egg dishes	12	10	9	15	15
Fat spreads[b] *of which:*	17	27	21	21	22
Butter	1	1	1	1	3
Reduced fat spread polyunsaturated (41-75% fat)	5	5	4	4	4
Reduced fat spread not polyunsaturated (41-75% fat)	9	19	13	13	11
Low fat spread polyunsaturated (18-39% fat)	1	3	2	3	4
Meat and meat products *of which:*	24	24	35	30	26
Bacon and ham	3	3	5	5	5
Beef, veal and dishes	3	3	5	6	4
Lamb and dishes	0	1	1	1	3
Pork and dishes	0	1	2	2	3
Coated chicken and turkey	2	1	2	0	0
Chicken, turkey and dishes	2	2	5	7	3
Liver and dishes	0	0	0	0	0
Burgers and kebabs	1	2	4	1	1

National Diet and Nutrition Survey: year 2, 3, 4 and 5 combined (2009/10 - 2012/13) Wales					
Food group[a]	**Age group** (years)				
	1.5-3	4-10	11-18	19-64	65+
Sausages	9	8	8	4	4
Meat pies and pastries	3	2	3	2	2
Other meat, meat products and dishes	1	1	1	2	2
Fish and fish dishes *of which:*	8	6	7	14	18
Oily fish	6	3	4	9	16
Vegetables and potatoes *of which:*	1	1	2	1	1
Vegetables (not raw) including vegetable dishes	0	0	1	1	0
Other potatoes, potato salads and dishes	1	0	1	1	0
Savoury snacks	0	0	0	0	0
Nuts and seeds	0	0	0	0	0
Fruit	0	0	0	0	0
Sugar, preserves and confectionery *of which:*	0	0	1	0	0
Chocolate confectionery	0	0	1	0	0
Non-alcoholic beverages[c] *of which:*	0	0	0	1	0
Soft drinks, not low calorie	0	0	0	1	0
Alcoholic beverages	0	0	0	0	0
Miscellaneous[d]	1	2	1	1	2
Savoury sauces, pickles, gravies and condiments	0	0	1	1	0
Commercial toddler foods	0	0	0	0	0
Average daily total vitamin D intake (µg)	1.9	2.0	2.2	2.9	3.2
Bases (unweighted)	*67*	*149*	*175*	*328*	*133*

a Food groups that contribute <0.5% to intake across all age/sex groups are excluded from the table. All other food groups are included.
b Some oils which are used as a condiment on bread or salads are included in this food group; however this food group does not include oils or fats used in cooking.
c Non-alcoholic beverages are reported as consumed with diluent water.
d Also includes nutrition powders and drinks.

Table 7: Average daily intake of vitamin D from all sources (including dietary supplements) and food sources for children aged 4-18 months, by age

Diet and Nutrition Survey of Infants and Young Children aged 4-18 months, 2011				
	Age group (months)			
	4-6	7-9	10-11	12-18
Vitamin D non-breastfed [a]				
Mean	10.0	8.9	7.7	3.9
Median	9.7	8.8	7.8	1.9
SD	2.9	3.1	3.5	3.9
Upper 2.5th percentile	17.1	16.0	15.7	14.0
Lower 2.5thpercentile	4.3	3.0	0.7	0.3
Vitamin D breastfed excluding breast milk[b]				
Mean	3.5	3.6	3.8	2.6
Median	2.3	3.1	3.2	1.5
SD	3.8	2.7	3.5	2.8
Upper 2.5th percentile	9.6	10.0	15.7	10.8
Lower 2.5th percentile	0.0	0.2	0.2	0.2
Food sources				
Vitamin D non-breastfed [a]				
Mean	9.8	8.7	7.5	3.5
Median	9.6	8.7	7.6	1.7
SD	2.7	2.9	3.4	3.5
Upper 2.5th percentile	14.8	15.2	15.6	12.0
Lower 2.5th percentile	4.3	3.0	0.7	0.3
Vitamin D breastfed excluding breast milk [b]				
Mean	3.0	3.2	2.7	1.8
Median	1.5	2.6	2.2	1.2
SD	3.5	2.6	2.3	1.7
Upper 2.5th percentile	9.6	8.5	8.2	5.7
Lower 2.5th percentile	0.0	0.2	0.1	0.2

a Vitamin D intake does not include values for breastfed children as the vitamin D content of breast milk is not known. Bases are: 240 for 4-6m, 489 for 7-9m, 381 for 10-11m and 1177 for 12-18m. Note breastfeeding status is defined by whether it was recorded in the 4-day diary.

b Vitamin D intake includes values for breastfed children excluding the contribution from breast milk (therefore excluding any exclusively breastfed children (n=2)) as the vitamin D content of breast milk is not known. Bases are 89 for 4-6m, 141 for 7-9m, 68 for 10-11m and 98 for 12-18m. Note breastfeeding status is defined by whether it was recorded in the 4-day diary.

Table 8: Average daily intake of vitamin D as a percentage of Reference Nutrient Intake (RNI), for children aged 4-18 months, by age

Diet and Nutrition Survey of Infants and Young Children aged 4-18 months, 2011					
		Age group (months)			
		4-6	**7-9**	**10-11**	**12-18**
		%			
All sources					
Non-breastfed[a]	Mean	117	127	111	55
	Median	115	126	111	27
	SD	34	44	50	55
Breastfed excluding breast milk[b]	Mean	41	52	54	37
	Median	27	44	45	21
	SD	44	39	51	40
Food sources					
Non-breastfed[a]	Mean	115	125	108	50
	Median	113	124	108	24
	SD	32	41	49	50
Breastfed excluding breast milk[b]	Mean	35	46	38	26
	Median	18	37	31	17
	SD	41	37	33	24
Bases (unweighted)		*329*	*630*	*449*	*1275*

a Vitamin D intake does not include values for breastfed children as the vitamin D content of breast milk is not known. Bases are: 240 for 4-6m, 489 for 7-9, 381 for 10-11m and 1177 for 12-18m. Note breastfeeding status is defined by whether it was recorded in the 4-day diary.

b Vitamin D intake includes values for breastfed children excluding the contribution from breast milk (therefore excluding any exclusively breastfed children (n=2)) as the vitamin D content of breast milk is not known. Bases are 89 for 4-6m, 141 for 7-9m, 68 for 10-11m and 98 for 12-18m. Note breastfeeding status is defined by whether it was recorded in the 4-day diary.

Table 9: Average daily intake of Vitamin D (µg), adults and children by age and sex (*1.5 years and over)*

UK National Diet and Nutrition Survey year 1, 2, 3 & 4 combined (2008/09 - 2011/12)															
Vitamin D (µg)	**Sex and age group (years)**														
	Boys			**Men**		**Girls**			**Women**		**Total**				
	4-10	11-18	**Total boys**	19-64	65+	4-10	11-18	**Total girls**	19-64	65+	1.5-3	4-10	11-18	19-64	65+
Intake from food sources only															
Mean	2.0	2.4	2.2	3.1	3.9	1.9	1.9	1.9	2.6	2.9	1.9	2.0	2.1	2.8	3.3
Median	1.9	2.1	2.0	2.5	3.2	1.7	1.6	1.7	2.1	2.5	1.4	1.8	1.8	2.3	2.7
SD	1.0	1.3	1.2	2.3	2.7	1.1	1.2	1.2	1.9	1.9	2.0	1.1	1.3	2.1	2.3
Upper 2.5th percentile	4.5	5.7	5.4	9.2	11.9	4.2	4.9	4.6	7.5	7.7	8.5	4.4	5.4	8.5	9.2
Lower 2.5th percentile	0.5	0.4	0.5	0.5	0.9	0.4	0.3	0.4	0.4	0.5	0.3	0.4	0.4	0.5	0.7
Intake from all sources (incl supplements)															
Mean	2.7	2.6	2.7	3.9	5.1	2.6	2.1	2.3	3.4	5.2	2.3	2.7	2.4	3.6	5.1
Median	2.1	2.2	2.2	2.7	3.7	1.9	1.7	1.8	2.5	3.5	1.5	2.0	1.9	2.6	3.6
SD	2.1	1.9	2.0	4.5	4.0	4.5	1.6	3.2	3.0	4.8	2.4	3.5	1.8	3.8	4.5
Upper 2.5th percentile	8.0	7.7	7.7	12.3	16.8	7.3	6.4	6.9	11.8	20.2	10.6	7.5	6.9	12.0	19.2
Lower 2.5th percentile	0.6	0.4	0.5	0.6	0.9	0.4	0.3	0.4	0.5	0.7	0.3	0.5	0.4	0.6	0.8
Bases (unweighted)	*665*	*744*	*1409*	*1126*	*317*	*612*	*753*	*1365*	*1571*	*436*	*604*	*1277*	*1497*	*2697*	*753*

Table 10: Average daily intake (µg) of vitamin D in Scotland, adults and children by sex and age *(1.5 years and over)*

National Diet and Nutrition Survey. Years 1, 2, 3 and 4 (combined) (2008/2009 – 2011/12): Scotland.															
Vitamin D (µg)	**Sex and age group** (years)														
	Boys			**Men**		**Girls**			**Women**		**Total**				
	4-10	11-18	**Total**	19-64	65+	4-10	11-18	**Total**	19-64	65+	1.5-3	4 -10	11-18	19 - 64	65+
Intake from food sources only															
Mean	2.0	2.4	2.2	3.1	3.5	2.0	1.8	1.9	2.4	2.9	1.8	2.0	2.1	2.7	3.2
Median	1.8	1.9	1.8	2.6	3.2	1.8	1.6	1.7	1.8	2.4	1.5	1.8	1.7	2.3	2.5
SD	1.2	1.5	1.4	2.2	2.4	1.0	1.2	1.1	1.7	1.9	1.4	1.1	1.4	2.0	2.1
Upper 2.5th percentile	5.3	6.8	5.9	9.5	9.8	4.4	5.3	4.6	7.1	7.4	7.0	4.5	5.8	7.9	9.0
Lower 2.5th percentile	0.5	0.7	0.5	0.8	0.6	0.6	0.3	0.4	0.4	0.7	0.5	0.5	0.4	0.5	0.6
Intake from all sources (including supplements)															
Mean	2.5	2.5	2.5	3.7	4.7	2.5	2.0	2.2	3.1	4.6	2.1	2.5	2.2	3.4	4.7
Median	1.9	2.0	2.0	2.9	3.9	1.9	1.7	1.7	2.2	3.1	1.5	1.9	1.8	2.5	3.3
SD	1.9	1.6	1.7	2.9	3.4	2.2	1.5	1.9	3.2	4.5	1.8	2.0	1.6	3.1	4.0
Upper 2.5th percentile	8.0	6.8	7.2	11.5	13.1	10.8	6.0	8.0	10.2	22.4	7.6	8.2	6.8	10.9	15.1
Lower 2.5th percentile	0.5	0.7	0.6	0.8	0.6	0.6	0.3	0.4	0.4	0.7	0.5	0.6	0.4	0.6	0.6
Bases (unweighted)	163	199	362	273	80	144	197	341	377	137	125	307	396	650	217

Table 11: Average daily intake of vitamin D (µg) in Northern Ireland, adults and children by sex and age *(1.5 years and over)*

National Diet and Nutrition Survey. Years 1, 2, 3 and 4 (combined) (2008/2009 – 2011/12): Northern Ireland.															
Vitamin D(µg)	**Sex and age group (years)**														
	Boys			**Men**		**Girls**			**Women**		**Total**				
	4-10	11-18	**Total**	19-64	65+	4-10	11-18	**Total**	19-64	65+	1.5-3	4 -10	11-18	19 - 64	65+
Intake from food sources only															
Mean	1.9	2.4	2.2	2.9	1.9	1.8	1.8	2.3	1.8	1.9	2.1	2.6	3.6	2.7	3.2
Median	1.7	2.3	1.9	2.4	1.6	1.6	1.6	2.0	1.5	1.6	1.8	2.2	2.9	2.3	2.5
SD	0.9	1.3	1.2	1.8	1.0	1.0	1.0	1.2	1.1	0.9	1.2	1.6	2.2	2.0	2.1
Upper 2.5th percentile	4.0	5.7	4.6	6.9	4.3	4.3	4.3	5.7	4.8	4.0	5.1	6.7	10.1	7.9	9.0
Lower 2.5th percentile	0.5	0.6	0.5	0.8	0.7	0.5	0.5	0.6	0.3	0.5	0.5	0.6	0.6	0.5	0.6
Intake from all sources (including supplements)															
Mean	2.4	2.6	2.5	3.4	2.5	2.0	2.2	3.0	1.9	2.4	2.3	3.2	5.7	3.4	4.7
Median	1.8	2.5	2.1	2.6	1.9	1.6	1.7	2.3	1.6	1.8	1.9	2.5	4.0	2.5	3.3
SD	1.8	1.7	1.7	2.8	1.8	1.4	1.6	2.4	1.5	1.8	1.5	2.6	4.8	3.1	4.0
Upper 2.5th percentile	7.4	7.2	7.3	11.1	6.8	6.0	6.4	8.7	6.4	7.1	6.8	10.6	19.3	10.9	15.1
Lower 2.5th percentile	0.5	0.6	0.5	0.9	0.7	0.5	0.5	0.6	0.3	0.5	0.5	0.7	0.8	0.6	0.6
Bases (unweighted)	*94*	*120*	*214*	*145*	*88*	*116*	*378*	*246*	*94*	*182*	*236*	*391*	*79*	*650*	*217*

Table 12: Average daily intake of vitamin D (µg) in Wales, adults and children by sex and age *(1.5 years and over)*

National Diet and Nutrition Survey. Years 2,3,4 and 5 (combined) (2009/10 – 2012/13): Wales															
Vitamin D (µg)	**Sex and age group** (years)														
	Boys			**Men**		**Girls**			**Women**		**Total**				
	4-10	11-18	**Total**	19-64	65+	4-10	11-18	**Total**	19-64	65+	1.5-3	4 -10	11-18	19 - 64	65+
Intake from food sources only															
Mean	2.0	2.3	2.2	3.4	3.4	2.0	2.1	2.0	2.4	3.1	1.9	2.0	2.2	2.9	3.2
Median	1.8	2.1	2.0	2.8	2.7	1.7	1.9	1.8	2.0	2.2	1.5	1.8	2.0	2.3	2.6
SD	1.0	1.6	1.4	2.4	2.2	1.1	1.1	1.1	1.5	2.5	1.6	1.0	1.4	2.1	2.4
Upper 2.5th percentile	3.8	5.1	5.1	9.1	9.0	4.3	4.5	4.3	6.5	9.9	7.4	4.3	4.8	8.1	9.1
Lower 2.5th percentile	0.5	0.3	0.3	0.4	0.7	0.4	0.4	0.4	0.5	0.7	0.4	0.5	0.3	0.5	0.7
Intake from all sources (including supplements)															
Mean	2.2	2.4	2.3	4.0	4.3	2.3	2.3	2.3	3.0	6.4	2.2	2.3	2.4	3.5	5.5
Median	1.8	2.2	2.1	3.3	3.6	1.8	1.9	1.9	2.1	3.2	1.7	1.8	2.1	2.5	3.6
SD	1.2	1.8	1.6	3.1	3.3	1.7	1.4	1.5	3.2	7.0	2.1	1.5	1.6	3.2	5.7
Upper 2.5th percentile	5.8	7.6	6.0	12.9	11.0	8.9	6.2	6.6	10.4	22.7	9.7	6.0	6.7	12.0	22.7
Lower 2.5th percentile	0.6	0.3	0.3	0.6	0.7	0.4	0.4	0.4	0.5	0.7	0.4	0.5	0.3	0.6	0.7

Table 13: Average daily intake of vitamin D as a percentage of RNI by age and sex *(1.5 years and over)*

UK National Diet and Nutrition Survey year 1-4 combined (2008/09 - 2011/12)				
Mean vitamin D intake (µg) as %of RNI	**Sex and age group** (years)*			
	Infants	**Men**	**Women**	**Total**
	1.5-3	65+	65+	65+
Food sources only				
Mean	27	39	29	33
Median	20	32	25	27
SD	29	27	19	23
All sources (including dietary supplements)				
Mean	32	51	52	51
Median	21	37	35	36
SD	34	40	48	45
Bases (unweighted)	*604*	*317*	*436*	*753*

***** *For Vitamin D, there are no RNIs set between ages 4 and 64 years; therefore % RNI is only expressed for those aged 1.5 to 3 years and those aged 65 years & over*

Table 14: Average daily intake of Vitamin D as a percentage of RNI in Scotland, by age and sex *(1.5 years and over)**

National Diet and Nutrition Survey. Years 1, 2, 3 and 4 (combined) (2008/2009 – 2011/12): Scotland				
Mean vitamin D intake as % of RNI	**Sex and age group** (years)*			
	Infants	**Men**	**Women**	**Total**
	1.5-3	65+	65+	65+
Food sources only				
Mean	26	35	29	32
Median	21	32	24	25
SD	19	24	19	21
All sources (including dietary supplements)				
Mean	30	47	46	47
Median	22	39	31	33
SD	26	34	45	40
Bases (unweighted)	*125*	*80*	*137*	*217*

**For Vitamin D, there are no RNIs set between ages 4 and 64 years; therefore % RNI is only expressed for those aged 1.5 to 3 years and 65 years and over.*

Table 15: Average daily intake of Vitamin D as a percentage of RNI in Northern Ireland, by sex and age *(1.5 y and over)**

National Diet and Nutrition Survey. Years 1,2,3 and 4 (combined) (2008/2009 – 2011/12): Northern Ireland.		
Mean vitamin D intake as % of RNI	**Age group** (years)	
	1.5-3	65+**
Food sources only		
Mean	25	36
Median	22	29
SD	16	22
All sources (including dietary supplements)		
Mean	28	57
Median	22	40
SD	21	48
Bases (unweighted)	*94*	*79*

***** *For Vitamin D, there are no RNIs set between ages 4 and 64 years; therefore % RNI is only expressed for those aged 1.5 to 3 years and 65 years and over.*

****** *Due to small cell sizes, participants aged 65 years and over have only been reported as males and females combined.*

Table 16: Average daily intake of vitamin D as a percentage of RNI in Wales, by sex and age *(1.5 years and over)**

National Diet and Nutrition Survey. Years 2, 3, 4 and 5 (combined) (2009/2010 – 2012/13):Wales				
Vitamin D	**Sex and age group** (years)			
Mean intake as % of RNI	**Infants**	**Men**	**Women**	**Total**
	1.5-3	65+	65+	65+
Food sources only				
Mean	27	34	31	32
Median	21	27	22	26
SD	22	22	25	24
All sources (including dietary supplements)				
Mean	32	43	64	55
Median	24	36	32	36
SD	30	33	70	57
Bases (unweighted)	*67*	*54*	*79*	*133*

**For Vitamin D, there are no RNIs set between ages 4 and 64 years; therefore % RNI is only expressed for those aged 1.5 to 3 years and 65 years and over.*

Table 17: Average daily intake of vitamin D from food sources (µg/day) in low income/materially deprived consumers by age and sex *(2 years and over)*

Low Income Diet and Nutrition Survey: 2003/05																
	Sex and age group (years)															
	Boys			**Men**					**Girls**			**Women**				
Intake from food sources	2-10	11-18	**Total boys**	19-34	35-49	50-64	65+	**Total men**	2-10	11-18	**Total girls**	19-34	35-49	50-64	65+	**Total women**
Mean	2.00	2.43	2.18	3.01	3.03	3.67	3.41	3.28	1.74	2.07	1.88	2.16	2.52	2.83	2.64	2.51
Median	1.78	2.03	1.88	2.54	2.70	3.00	2.88	2.81	1.45	1.87	1.60	1.86	1.93	2.34	2.24	2.04
SD	1.30	1.44	1.37	2.09	1.87	2.81	2.29	2.30	1.15	1.14	1.15	1.51	3.25	2.13	1.70	2.26
Upper 2.5th percentile	4.76	7.00	5.14	10.03	7.53	10.03	9.71	9.52	4.09	5.04	4.85	6.05	7.77	9.23	6.67	7.09
Lower 2.5th percentile	0.08	0.79	0.22	0.64	0.33	0.46	0.65	0.60	0.12	0.31	0.25	0.44	0.35	0.25	0.37	0.41
Bases (unweighted)	*239*	*200*	*439*	*194*	*226*	*258*	*268*	*946*	*278*	215	493	483	494	336	537	1850

Table 18: Average daily intake of vitamin D for low income materially deprived consumers as a percentage of RNI, by age and sex (1.5 years and over)

Low Income Diet and Nutrition Survey 2003/05				
	Sex and age group (years)*			
	Males		**Females**	
Food sources only	2-10	65+	2-10	65+
Mean	22	34	22	26
Median	21	29	20	22
Bases (unweighted)	*239*	268	278	537

* For Vitamin D, there are no RNIs set between ages 4 and 64 years; therefore % RNI is only expressed for those aged 1.5 to 3 years and 65 years and over.

Serum/plasma 25(OH)D concentrations in the UK

Table 19: Infants and young children 4-18 months

Diet and Nutrition Survey of Infants and Young Children 2011		
	Age group (months)	
Serum 25-hydroxyvitamin D (nmol/L)*	5-11	12 +
Mean	68.6	64.3
Median	67.6	62.9
SD	25.2	24.3
Upper 2.5th percentile	110.0	122.0
Lower 2.5th percentile	12.1	26.2
% below 25 nmol/L	*6%*	*2%*
Bases (unweighted)	*166*	*300*

**Blood samples collected February to August*

Table 20: UK - Adults and children by age and sex *(1.5 years and over)*

UK National Diet and Nutrition Survey years 1-4 combined (2008/09-2011/12)													
	Sex and age group (years)												
	Boys		**Men**		**Girls**		**Women**		**All**				
Plasma 25-hydroxyvitamin D (nmol/L)	4-10	11-18	19-64	65+	4-10	11-18	19-64	65+	1.5-3	4-10	11-18	19-64	65+
Mean	52.3	44.9	43.5	47.0	48.0	41.1	47.3	42.5	[58.1]	50.2	43.0	45.4	44.5
Median	51.7	42.8	39.0	45.5	49.1	38.3	44.5	41.4	[51.0]	50.4	40.7	41.8	43.1
SD	23.96	23.75	23.87	22.47	22.21	21.08	25.61	20.18	[26.75]	23.16	22.51	24.84	21.29
Upper 2.5th percentile	106.0	100.0	92.4	94.8	108.0	87.5	106.0	87.1	[105.0]	106.0	96.3	104.0	94.8
Lower 2.5th percentile	16.1	10.4	7.1	12.3	14.3	7.1	10.2	11.8	[17.4]	14.3	7.1	7.1	12.1
% below 25 nmol/L	12.3	19.7	24.0	16.9	15.6	24.4	21.7	24.1	[7.5]	13.9	22.0	22.8	21.0
Bases (unweighted)	*129*	*273*	*551*	*140*	*108*	*250*	*770*	*198*	*[42]*	*237*	*523*	*1321*	*338*

Table 21: Scotland - adults and children, by age and sex *(4 years and over)*

National Diet and Nutrition Survey 2008/09-2011/12 Scotland										
	Sex and age group (years)[a]									
	Boys	**Men**		**Girls**	**Women**		**Total**			
Plasma 25-hydroxyvitamin D (nmol/L)	11-18	19-64	65+	11-18	19-64	65+	4-10	11-18	19-64	65+
Mean	38.0	39.9	[43.4]	36.5	40.2	40.2	47.0	37.3	40.1	41.5
Median	30.6	35.7	[40.5]	36.5	33.7	35.2	44.9	32.8	34.7	38.9
SD	22.63	22.77	[22.67]	15.55	24.64	23.11	14.82	19.43	23.68	22.79
Upper 2.5th percentile	100.0	91.9	[90.6]	72.8	104.0	104.5	81.9	86.9	91.9	90.6
Lower 2.5th percentile	7.1	7.1	[12.4]	7.1	7.1	7.1	16.6	7.1	7.1	7.1
% below 25 nmol/L[e]	29.0	31.7	[26.3]	23.0	33.3	31.6	9.2	26.1	32.5	29.4
Bases (unweighted)	*73*	*137*	*[34]*	*64*	*182*	*66*	*58*	*137*	*319*	*100*

Table 22: Northern Ireland - adults and older children by age and sex *(11 years and over)*

2008/09-2011/12 Northern Ireland							
	Sex and age group *(years)*						
	Boys	**Men**	**Girls**	**Women**	**Total**		
Plasma 25-hydroxyvitamin D (nmol/L)	11-18	19-64	11-18	19-64	11-18	19-64	65+
Mean	[38.5]	36.8	38.7	42.3	38.6	39.4	[45.1]
Median	[37.6]	31.4	35.4	34.5	35.4	33.1	[46.6]
SD	[20.04]	19.34	21.78	27.24	20.82	23.57	[19.21]
Upper 2.5th percentile	[90.1]	82.6	93.3	113.0	93.3	97.7	[74.0]
Lower 2.5th percentile	[11.5]	12.0	14.1	10.6	11.8	11.7	[7.1]
% below 25 nmol/L	[24.8]	35.6	34.5	33.0	29.5	34.3	[18.7]
Bases (unweighted)	*[41]*	*98*	*51*	*121*	*92*	*219*	*[34]*

Table 23: Wales - adults and older children by age and sex (*11 years and over)*

2009/10-2012/13 Wales					
	Sex and age group (years)				
	Men	**Women**	**Total**		
Plasma 25-hydroxyvitamin D (nmol/L)	19-64	19-64	11-18	19-64	65+
Mean	50.9	42.5	42.9	46.4	43.0
Median	53.8	39.5	40.0	43.3	40.0
SD	26.79	19.42	21.63	23.42	19.09
Upper 2.5th percentile	94.7	89.5	95.9	94.7	81.9
Lower 2.5th percentile	13.5	10.8	15.2	12.8	11.1
% below 25 nmol/L	19.8	15.8	23.0	17.7	16.5
Bases (unweighted)	*62*	*105*	*53*	*167*	*50*

Table 24: England – adults 65 years and over

Health Survey for England 2005															
Serum 25-hydroxyvitamin D (nmol/L)	**Sex and age group (years)**														
	Men					**Women**					**All**				
	65-69	70-74	75-79	80-84	85+	65-69	70-74	75-79	80-84	85+	65-69	70-74	75-79	80-84	85+
Mean	53.3	55.6	51.6	48.6	48.2	52.4	51.7	43.5	44.8	42.3	52.8	53.5	47.1	46.2	44.6
Median	52.5	54.0	47.7	45.0	48.0	49.0	49.0	40.0	43.0	38.0	50.1	51.0	44.0	44.0	41.0
SD	21.4	23.8	23.8	20.0	19.5	23.8	21.7	20.2	19.3	24.0	22.6	22.8	22.2	19.6	22.5
Upper 2.5th percentile	101.5	109.0	111.4	95.8	88.7	107.4	98.1	88.0	84.0	116.2	101.9	101.0	102.0	87.6	102.4
Lower 2.5th percentile	17.0	14.7	14.6	18.0	11.9	15.9	17.0	12.0	13.0	11.0	16.0	16.0	13.0	15.0	11.3
% below 25 nmol/L	*8*	*8*	*12*	*7*	*8*	*7*	*11*	*19*	*16*	*22*	7	9	16	13	17
% below 15 nmol/L	*2*	2	2	0	3	2	1	4	3	8	2	1	3	2	6
Bases (unweighted)	*295*	*240*	*185*	*113*	*74*	*316*	*278*	*240*	*205*	*119*	*610*	*518*	*425*	*318*	*193*

Table 25: UK - low income/materially deprived consumers (aged 8 years and over)

Low Income Diet and Nutrition Survey 2004/05												
Serum 25-hydroxyvitamin D (nmol/L)	**Sex and age group (years)**											
	Boys		**Men**				**Girls**		**Women**			
	8-10	11-18	19-34	35-49	50-64	65+	8-10	11-18	19-34	35-49	50-64	65+
Mean	[65.9]	43.5	44.9	43.2	45.8	52.8	[52.2]	39.6	48.5	48.7	43.2	44.2
Median	[61.0]	41.0	37.5	39.0	38.0	44.0	[51.2]	33.0	44.0	44.0	39.0	40.0
SD	[18.0]	16.8	22.6	21.2	26.4	35.4	[19.4]	21.8	26.0	25.4	22.7	20.7
Upper 2.5th percentile	[109.0]	80.2	106.0	84.5	107.0	193.0	[86.0]	92.0	108.0	103.0	117.0	95.0
Lower 2.5th percentile	[40.0]	12.0	15.0	12.4	14.2	17.0	[20.0]	11.0	12.0	10.0	14.0	9.1
% below 25 nmol/L	*[0]*	*8*	*18*	*24*	*25*	*14*	*[16]*	*23*	*19*	*14*	*24*	*14*
Bases (unweighted)	*8*	*37*	*65*	*95*	*133*	*145*	*15*	*45*	*200*	*237*	*181*	*258*

Table 26: UK - by month blood sample taken, adults and children by age *(1.5 y and over)*

National Diet and Nutrition Survey: years 1-4 combined (2008/09-2011/12)					
Plasma 25-hydoxyvitamin D*	**Age group** (years)				
	1.5-3[a]	4-10	11-18	19-64	65+
January-March[1,b]					
Mean		37.2	31.5	34.8	40.5
Median		32.9	28.1	29.4	36.7
SD		17.56	18.77	22.91	22.91
% below 25 nmol/L		31.4	40.0	39.3	29.3
April-June[2,b]					
Mean		[47.2]	43.5	44.2	44.9
Median		[48.9]	41.2	40.4	38.2
SD		[18.60]	19.27	24.39	21.89
% below 25 nmol/L		[8.2]	12.7	24.4	21.3
July-September[3,b]					
Mean		66.0	52.3	57.5	50.5
Median		60.8	50.3	56.3	48.7
SD		22.66	21.39	23.42	18.31
% below 25 nmol/L		1.7	13.4	8.4	3.6
October-December[4,b]					
Mean		50.2	44.3	45.6	43.7
Median		52.9	37.3	41.0	42.6
SD		23.14	26.62	22.73	19.94
% below 25 nmol/L		11.7	24.3	16.9	25.7
Bases (unweighted)					
[1] January-March	*[12]*	*68*	*125*	*345*	*106*
[2] April-June	*[8]*	*[48]*	*152*	*369*	*85*
[3] July-September	*[8]*	*59*	*136*	*341*	*75*
[4] October-December	*[14]*	*62*	*110*	*266*	*72*

* 0.0% represents no cases in this dataset.

a Due to cell sizes for those aged 1.5 to 3 years being below 30, data has not been presented for children aged 1.5 to 3 years.

b Due to limited cell sizes, the 2.5th and 97.5th percentiles have not been presented.

Table 27: England - adults by season of interview *(aged 16 years and over)*

Health Survey for England 2010				
	Sex and season of interview*			
Serum 25-hydroxyvitamin D (nmol/L)	**Summer July - Sept**	**Autumn Oct - Dec**	**Winter Jan - March**	**Spring April – June**
Mean	60.1	39.4	33.1	45.2
Median	58.5	36.5	27.0	43.0
% below 25 nmol/L	*6.9*	*27.4*	*42.2*	18.2
Bases (unweighted)	*998*	*971*	*1,557*	*1,220*

**Blood sample taken shortly after interview*

Table 28: Scotland - adults by season of interview *(aged 16 years and over)*

Scottish Health Survey 2010-2011 combined				
	Sex and season of interview*			
Plasma 25-hydroxyvitamin D (nmol/L)	**Summer** July - Sept	**Autumn** Oct - Dec	**Winter** Jan - March	**Spring** April – June
Mean	51.3	34.9	27.9	36.1
SE	1.9	1.4	1.2	1.3
SD	26.2	19.7	16.9	18.8
% below 25nmol/L	*17*	*32*	*54*	30
95% CI %	*12-23*	*26-39*	*47-62*	*25-36*
Bases (unweighted)	*364*	*320*	*413*	*356*

*** Blood sample taken shortly after interview*

Table 29: English regions by season - adults *(aged 16 years and over)*

Health Survey for England 2010												
	Summer: July - September			**Autumn:** October - December			**Winter:** January - March			**Spring:** April - June		
Plasma 25-hydroxyvitamin D (nmol/L)	Midlands & North	South incl. London	South excl. London	Midlands & North	South incl. London	South excl. London	Midlands & North	South incl. London	South excl. London	Midlands & North	South incl. London	South excl. London
% below 25 nmol/L	*6*	*7*	*5*	*29*	*26*	*21*	*46*	*38*	*35*	*22*	*14*	12
Bases	*504*	*494*	*391*	*517*	*454*	*344*	*837*	*720*	*570*	*645*	*575*	*469*

% below 25 mmol/L in London:

Summer 16%;
Autumn 47%;
Winter 55%;
Spring 6%

Table 30: Pregnant women in North West London, by season

Multi-ethnic sample of pregnant women in North West London 2008-2009					
	Month blood sample taken				
Serum 25-hydroxyvitamin D (nmol/L)	July - September	October - December	January - March	April - June	Overall
Median	38.0	38.0	26.0	32.0	35.0
Inter quartile range	22, 76	18, 66	12, 48	20, 60	19, 64
% below 25nmol/L	*29*	*36*	*49*	*34*	*36*
Bases (unweighted)					346

Source: McAree T, Jacobs B, Manickavasagar T *et al.* Vitamin D deficiency in pregnancy - still a public health issue. *Matern Child Nutr.* 2013. **9**; 23-30.

Table 31: Pregnant women in Southampton

Pregnant women taking part in Southampton Women's Survey	
Serum 25-hydroxyvitamin D (nmol/L)**	
Median	62.0
Inter quartile range	43-89
% below 75nmol/L	*63.4*
% below 50nmol/L	35.1
Bases (unweighted)	*977*

*Mean age 30.4 years

**Measured at 34 weeks gestation. Blood samples taken throughout year. Season of sampling was most important predictor of higher 25(OH)D concentration

Source: Crozier SR, Harvey NC, Inskip HM *et al Vi.*tamin D status in pregnancy is associated with adiposity in the offspring: findings from the Southampton Women's Survey. *AJCN.* 2012. 96; 57-63

Table 32: Pregnant women* in South West England, by trimester

ALSPAC study 1991 / 1992			
	1st trimester	**2nd trimester**	**3rd trimester**
Serum 25-hydroxyvitamin D (nmol/L)**			
Median	55.1	60.1	67.4
Inter quartile range	40.7 - 74.1	41.4 - 83.4	46.8 - 93.0
% below 50 nmol/L			*34%*
% below 27.5 nmol/L			6%
Bases (unweighted)	*1035*	*879*	*2,046*

*Mainly white European; mean age 29 years.
**Blood samples collected throughout year.

Source: Lawlor DA, Wills AK, Fraser A *et al.* Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. *Lancet*. 2013; 381(9884): 2176-83.

Table 33: England – by ethnicity, adults *(aged 16 years and over)*

Health Survey for England 2010					
	Ethnic Group				
Serum 25-hydroxyvitamin D (nmol/L)	White	Mixed	Asian	Black	Other
Mean	45.8	[31]	20.5	27.7	[22.4]
Median	43.0	[24]	15.0	23.0	[18]
% below 25 nmol/L	*21*	*[52]*	*74.8*	*54.2*	*[63.9]*
Bases (unweighted)	*3,548*	*[25]*	*135*	*72*	*[36]*

Table: 34: Asian children aged 2 years in England

Asian Infant Feeding Survey 1996			
	Ethnic group		
Plasma 25-hydroxyvitamin D (nmol/L)*	**Bangladeshi**	**Pakistani**	**Indian**
Mean	42.1	36.2	42.2
Median	37.5	30.0	37.5
SD	21.30	19.60	22.50
Upper 2.5th percentile	91.2	92.5	102.5
Lower 2.5th percentile	16.5	14.5	14.7
% below 25 nmol/L	*20*	*34*	*25*
% below 20 nmol/L	*13*	*18*	*13*
% below 12.5 nmol/L	*0*	*0*	*0*
Bases (unweighted)	*139*	*200*	*279*

*Blood samples collected October/November

Table 35: Women in Southern England, by ethnicity and season *(aged 19-70 years)*

Women living in Southern England								
	Ethnicity and season blood sample taken							
	Summer 2006		**Autumn 2006**		**Winter 2006/07**		**Spring 2007**	
Serum 25-hydroxyvitamin D (nmol/L)*	Asian	White	Asian	White	Asian	White	Asian	White
Mean	26.8	67.9	21.4	58.3	20.2	43.7	21.9	48.6
Median	24.4	65.1	18.7	54.0	16.1	40.7	19.0	44.3
SD	10.20	23.10	10.10	21.60	10.40	16.20	10.10	20.90
% below 25nmol/L	*52.5*	*0.4*	*80*	*1.8*	*75.4*	*9.7*	72.7	6.7
Bases (unweighted)	*86*	*279*	*77*	*247*	*71*	*224*	*70*	*223*

*The same women gave blood samples in each season

Source: S Lanham-New, *personal communication*, 2013.

Table 36: Scotland - by Body Mass Index (BMI) *(aged 16 years and over)*

Scottish Health Survey 2010-2011 combined			
Plasma 25-hydroxyvitamin D (nmol/L)	**Body Mass Index**		
	Less than 25	25 to less than 30	30 and over
Mean Vitamin D	41	38.5	33.3
SE of mean	1.8	1.1	1.1
SD	25.7	21.7	18.4
% below 25 nmol/L (95% CI)	33 (27-40)	28 (24-33)	38 (32-44)
Bases (unweighted):	*412*	*515*	*400*

Glossary

1α-hydroxycholecalciferol	A synthetic analogue of 1,25-dihydroxyvitamin D.
1,25-dihydroxyvitamin D ($1,25(OH)_2D$)	The main active metabolite of vitamin D in the body. Produced in the kidney from 25-hydroxyvitamin D.
7-dehydrocholesterol (7-DHC)	A sterol found in the skin of humans and animals that is converted to vitamin D_3 by the action of UVB rays in sunlight.
25-hydroxyvitamin D (25(OH)D)	A metabolite of vitamin D produced in the liver from vitamin D. Circulates in the blood and is a marker of exposure to vitamin D, reflecting vitamin D supply from cutaneous synthesis and the diet.
Alfacalcidol	Another name for 1α-hydroxycholecalciferol.
Antigen	Substance (usually foreign) that prompts the generation of antibodies and induces an immune response.
B cell	See *lymphocyte.*
Biomarker	A biochemical, physiological, or other indicator that is objectively measured to assess effects of normal biological processes, progress of a disease, illness, condition or response to an intervention.
Bone health indices	Biochemical markers that influence, predict or are associated with the processes of bone formation, bone resorption or other facets of bone metabolism.
Bone mineral content (BMC)	The mass of bone mineral in a skeletal unit (generally measured in grams (g), occasionally in g/cm cross-sectional width).
Bone mineral density (BMD)	The density of bone mineral in a skeletal unit (g/cm^3). When measured by single or dual-energy X-ray techniques it represents the mass of bone mineral measured within a scanned area (g/cm^2) and is not a true density measurement.
Bone modelling	The process of skeletal growth and development during childhood in which bone formation and resorption take place on different bone surfaces.
Bone remodelling	Lifelong process of bone replacement and repair in which old bone is broken down (resorption) and replaced by new bone (formation or ossification) on the same bone surface.
Calcidiol	Another name for 25-hydroxyvitamin D.
Calcifidiol	Another name for 25-hydroxyvitamin D.

Calcitonin	A peptide hormone, produced by the thyroid gland, involved in regulation of calcium homeostasis.
Calcitriol	Another name for 1,25-dihydroxyvitamin D.
Cholecalciferol	Another name for vitamin D_3.
Confounding variable (confounder)	Associated independently with both the health outcome under study and the exposure of interest. The effect of an association between an exposure and outcome is distorted by the presence of one or more (confounding) variables.
Dendritic cells	Antigen-presenting cells of the immune system. They express receptors that enhance the uptake of antigens, which they present to T cells (see *lymphocyte)*.
Dietary Reference Values (DRV)	DRVs describe the distribution of nutrient and energy requirements in a population. They comprise 3 estimates: **Estimated Average Requirement (EAR):** half of a group in a population will need more than this amount and half will need less; **Reference Nutrient Intake (RNI)**: the intake that will be adequate to meet the needs of 97.5% of the population; **Lower Reference Nutrient Intake (LRNI):** the intake which will meet the needs of only 2.5% of the population.
Endocytosis	Uptake of extracellular material by invagination of the cell membrane which then breaks off to form a vesicle enclosing the material.
Enterocyte	Epithelial cell of the small intestine and colon, specialised for uptake of the products of digestion in the lumen and transport into the bloodstream.
Ergocalciferol	Another name for vitamin D_2.
Ergosterol	A plant sterol that is converted into vitamin D_2 by ultraviolet radiation.
Erythema	Reddening of the skin as a result of increased cutaneous blood flow. May be caused by sunburn.
Femur	Longest and strongest bone in the human skeleton. Located in the thigh, extending from hip to knee.
Estimated Average Requirement (EAR)	See *Dietary Reference Values*.
Fibroblast growth factor 23 (FGF23)	A hormone that down-regulates 1,25 $(OH)_2D$ synthesis in the kidney.
Fortification	Addition of nutrients to foods during the manufacturing process.

Genetic polymorphism	Natural variation in a gene, DNA sequence or chromosome.
Genome	Full DNA sequence of an organism.
Genotype	Genetic constitution of an individual as distinct from its expressed features.
Half-life	Time required for concentration of a substance in the body to decrease by half.
Heterogeneous	Varied in content; composed of different parts.
Homeostasis	The process by which the internal systems of the body maintain a balance despite external conditions.
Homogeneous	Having parts which are all similar or which consist of only one substance.
Hypercalciuria	Elevated calcium in the urine. Defined as urinary calcium excretion > 250 mg/d in women and 275-300 mg/d in men.
Hyperparathyroidism	Over-activity of the parathyroid glands that results in high concentration of parathyroid hormone. This leads to weakening of the bones through bone resorption to release calcium from the bone.
Lower Reference Nutrient Intake (LRNI)	See *Dietary Reference Values*.
Lymphocyte (B and T)	White blood cells that form part of the body's immune system. B cells produce antibodies that neutralise foreign bodies like viruses and bacteria; T cells produce substances that directly and indirectly induce the death of infected cells.
Macrophage	A type of white blood cell that forms part of the body's immune system. Macrophages engulf invading antigens and ultimately stimulate production of antibodies against the antigen.
Minimal erythemal dose (MED)	Minimum dose of ultraviolet radiation required to produce a just measurable degree of sunburn or redness. Varies with each individual.
Metabolic bone disease	Bone abnormalities usually caused by an imbalance of minerals (calcium and phosphorus) or vitamin D in the body.
Metabolites	Intermediates and products of chemical reactions in the body.
Monocytes	A type of white blood cell that, in response to inflammation, differentiates into macrophages and dendritic cells to elicit an immune response.
Osteoblast	Bone cell that synthesises osteoid and facilitates its calcification as part of the processes of skeletal development, maintenance and repair.

Osteoclast	Bone cell that resorbs bone tissue as part of the processes of skeletal development, maintenance and repair.
Osteocyte	Bone cell that acts as a mechanosensor and plays a role in activation of osteoblasts and osteoclasts for bone formation and resorption.
Osteoid	Uncalcified, pre-bone tissue produced by osteoblasts.
Osteomalacia	A skeletal disorder that develops as result of vitamin D deficiency. Causes severe aching in bones and muscles and muscle weakness. Caused by impairment in mineralisation phase of bone remodelling, resulting in a lower ratio of bone mineral to osteoid than normal. Kidney or liver damage, which interferes with vitamin D metabolism can also cause osteomalacia.
Osteoporosis	A progressive skeletal disorder characterised by reduced bone strength due to loss of bone mass and deterioration in micro-architecture of bone, where the ratio of bone mineral to osteoid is normal. Leads to increased bone fragility and risk of fracture.
Parathyroid hormone (PTH)	Hormone secreted by the parathyroid glands. Involved in regulation of calcium homeostasis.
Phenotype	Observable physical and/or biochemical characteristics of the expression of a gene under a particular set of environmental conditions.
Reference Nutrient Intake (RNI)	See *Dietary Reference Values*.
Residual confounding	Occurs when one or more confounders (see above) have not been adequately controlled for in analysis or where such variables cannot be identified.
Reverse causation	Describes a situation in which a supposed outcome precedes and causes an exposure; type of bias in observational studies.
Rickets	A disorder of the growth plates of infants and children that affects skeletal development. The most common cause is insufficient vitamin D (vitamin D deficiency rickets), calcium or phosphate during growth, which leads to pain, softening and weakening of the bones and characteristic deformities of the long bones.
Safe intake	Set for some nutrients if there are insufficient reliable data to set dietary reference values. Represents a level or intake range at which it is considered that there is no risk of deficiency and below a level where there is a risk of undesirable effects.
Sensitivity analysis	A repeat of a meta-analysis of a group of studies, which excludes studies that may have affected the results of the meta-analysis through poor study quality or uncertainty about adherence to inclusion criteria.

T cell	See *lymphocyte*.
Tolerable upper intake level (UL)	The highest average daily intake of a nutrient that is likely to pose no risk of adverse effects to almost all individuals in the general population. It applies to chronic intakes on a chronic basis among free-living persons. As intake increases above UL, the potential risk of adverse effects may increase.
Trochanter	One of two protrusions at the top of the femur connecting to the hip bone: the greater (major) trochanter located on proximal and outside part of femur; the lesser (minor) trochanter located on proximal & inside part of femur. Points at which hip and thigh muscles attach.
Ultraviolet radiation (UVR)	UVR is part of the electromagnetic spectrum emitted by the sun with wavelengths of around 100-400 nm. Sub-regions of UVR spectrum have been defined according to transmission in human tissue and potential health effects: UVA (315-400 nm), UVB (28—315 nm) and UVC (100-280 nm).
Vitamin D_2	Also known as ergocalciferol. Formed in fungi and yeast by UVB exposure of the steroid, ergosterol.
Vitamin D_3	Also known as cholecalciferol. Synthesised in skin of humans from 7-dehydrocholesterol by the action of solar UVB radiation.
Ward's triangle	Region of femoral neck with lowest density and area of initial bone loss in older people.