DRAFT SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for vitamin D[1]

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA)[2,3]

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ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) derived Dietary Reference Values (DRVs) for vitamin D. The Panel considers that serum 25(OH)D concentration, which reflects the amount of vitamin D attained from both cutaneous synthesis and dietary sources, can be used as biomarker of vitamin D status in adult and children populations. The Panel notes that the evidence on the relationship between serum 25(OH)D concentration and musculoskeletal health outcomes in adults, infants and children, and adverse pregnancy-related health outcomes, is widely variable. The Panel considers that Average Requirements and Population Reference Intakes for vitamin D cannot be derived, and therefore defines Adequate Intakes (AIs), for all population groups. Taking into account the overall evidence and uncertainties, the Panel considers that a serum 25(OH)D concentration of 50 nmol/L is a suitable target value for all population groups, in view of setting the AIs. For adults, an AI for vitamin D is set at 15 µg/day, based on a meta-regression analysis and considering that, at this intake, most of the population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L. For children aged 1–17 years, an AI for vitamin D is set at 15 µg/day, based on the meta-regression analysis. For infants aged 7–11 months, an AI for vitamin D is set at 10 µg/day, based on trials in infants. For pregnant and lactating women, the Panel sets the same AI as for non-pregnant non-lactating women, i.e. 15 µg/day. The Panel underlines that the meta-regression was done on data collected under conditions of minimal cutaneous vitamin D synthesis. In the presence of cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may even be zero.

KEY WORDS

vitamin D, 25(OH)D, UV-B irradiation, musculoskeletal health outcomes, meta-regression, Adequate Intake, Dietary Reference Value

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on Dietary Reference Values (DRV) for the European population, including vitamin D.

Vitamin D belongs to the fat-soluble vitamins. It is the generic term for ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), which are formed from their respective provitamins, ergosterol and 7-dehydrocholesterol (7-DHC), following a two step-reaction involving ultraviolet-B (UV-B) irradiation and subsequent thermal isomerisation. Vitamin D_2 and vitamin D_3 are present in foods and dietary supplements. Vitamin D_3 is also synthesised endogenously in the skin following exposure to UV-B irradiation.

During summer months, or following exposure to artificial UV-B irradiation, the synthesis of vitamin D_3 in the skin may be the main source of vitamin D. Dietary intake of vitamin D is essential in case endogenous synthesis, due to insufficient UV-B exposure, is lacking or insufficient. Factors affecting the synthesis of vitamin D_3 in the skin include latitude, season, ozone layer and clouds (absorbing UV-B irradiation), surface characteristics (reflecting UV-B irradiation), time spent outdoors, use of sunscreens, clothing, skin colour, and age. The Panel notes that sun exposure may contribute a considerable and varying amount of vitamin D available to the body and therefore considers that the association between vitamin D intake and status, for the purpose of deriving DRVs for vitamin D, should be assessed under conditions of minimal endogenous vitamin D synthesis. Vitamin D from dietary sources is absorbed throughout the small intestine. The Panel considers that the average vitamin D absorption from a usual diet is about 80% and limited data are available on the effect of the food or supplement matrix on absorption of vitamin D (vitamin D_2 or vitamin D_3).

In the body, within hours of ingestion or synthesis in the skin, vitamin D is either converted into its biologically active metabolite 1,25$(OH)_2$D or delivered to the storage tissues (as either vitamin D or its metabolites). The first step of the activation occurs in the liver, where vitamin D is hydroxylated to 25(OH)D, while the second step occurs primarily in the kidneys, where 25(OH)D is hydroxylated to 1,25$(OH)_2$D. Vitamin D, 1,25$(OH)_2$D and 25(OH)D are transported in the blood bound mainly to the vitamin D-binding protein (DBP). Of the two metabolites of vitamin D, 25(OH)D is the major circulating form, with a longer half-life, of about 13-15 days. 25(OH)D is taken up from the blood into many tissues, including in the adipose tissue, muscle and liver for storage.

After its release from DBP to tissues, 1,25$(OH)_2$D exerts, in association with the intracellular vitamin D receptor (VDR), important biological functions throughout the body. In the intestine, it binds to VDR to facilitate calcium and phosphorus absorption. In the kidney, it stimulates the parathyroid hormone (PTH)-dependent tubular reabsorption of calcium. In the bone, PTH and 1,25$(OH)_2$D interact to activate the osteoclasts responsible for bone resorption. In addition, 1,25$(OH)_2$D suppresses the PTH gene expression, inhibits proliferation of parathyroid cells, and is involved in cell differentiation and antiproliferative actions in various cell types. Both 25(OH)D and 1,25$(OH)_2$D are catabolised before elimination and the main route of excretion is via the faeces.

Vitamin D deficiency leads to impaired mineralisation of bone due to an inefficient absorption of dietary calcium and phosphorus, and is associated with an increase in PTH. Clinical symptoms of vitamin D deficiency manifest as rickets in children, and osteomalacia in adults.

The Panel reviewed possible biomarkers of vitamin D intake and/or status, namely serum concentration of 25(OH)D, free 25(OH)D, 1,25$(OH)_2$D and PTH concentration, markers of bone formation and bone turnover. In spite of the high variability in 25(OH)D measurements obtained with different analytical methods, the Panel nevertheless concludes that serum 25(OH)D

concentration, which reflects the amount of vitamin D attained from both cutaneous synthesis and dietary sources, can be used as biomarker of vitamin D status in adult and children populations. Serum 25(OH)D concentration can also be used as biomarker of vitamin D intake in a population with low exposure to UV-B irradiation.

In consideration of the various biological functions of $1,25(OH)_2D$, the Panel assessed the available evidence on the relationship between serum 25(OH)D concentration and several health outcomes, to evaluate whether they might inform the setting of DRVs for vitamin D. The Panel first considered the available evidence on serum 25(OH)D concentration and musculoskeletal health outcomes, i.e. bone mineral density (BMD)/bone mineral content (BMC) and calcium absorption in adults and infants/children, risk of osteomalacia, fracture risk, risk of falls/falling, muscle strength/muscle function/physical performance in adults, and risk of rickets in infants/children. The Panel then reviewed data on the relationship between maternal serum 25(OH)D concentration and health outcomes in pregnancy (risk of pre-eclampsia, of small for gestational age and of pre-term birth, and indicators of bone health in infants) and lactation. The Panel took as starting point the results and conclusions from the most recent report on DRVs for vitamin D by the Institute of Medicine (IOM) that was based on two systematic reviews. The Panel also considered an update of one of these two systematic reviews, as well as two recent reports from DRV-setting bodies, and undertook a separate literature search to identify primary intervention and prospective observational studies in healthy subjects that were published after the IOM report. As a second step, the Panel considered available evidence on several other non-musculoskeletal health outcomes (e.g. cancer or cardiovascular diseases), based on the reports and reviews mentioned above without undertaking a specific literature search of primary studies. The Panel considers that the available evidence on serum 25(OH)D concentration and musculoskeletal health outcomes and pregnancy-related health outcomes is suitable to set DRVs for vitamin D for adults, infants, children, and pregnant women, respectively. However, the Panel considers that there is no evidence for a relationship between serum 25(OH)D concentration and health outcomes of lactating women that may be used to set a DRV for vitamin D, and that the available evidence on non-musculoskeletal-related health outcomes is insufficient to be used as criterion for setting DRVs for vitamin D.

The Panel notes that data on the relationship between serum 25(OH)D concentration and adverse musculoskeletal or pregnancy-related health outcomes are widely variable. However, taking into account the overall evidence and uncertainties, the Panel considers that, overall, for adults, infants and children, there is evidence for an increased risk of adverse musculoskeletal health outcomes at serum 25(OH)D concentrations below 50 nmol/L. The Panel also considers that there is evidence for an increased risk of adverse pregnancy-related health outcomes at serum 25(OH)D concentrations below 50 nmol/L.

The Panel assessed the available evidence on the relationship between vitamin D intake and musculoskeletal health outcomes to evaluate whether they might inform the setting of DRVs for vitamin D. The Panel notes that these studies usually do not provide information on the habitual dietary intake of vitamin D, and the extent to which cutaneous vitamin D synthesis has contributed to the vitamin D supply (and thus may have confounded the relationship between vitamin D intake and the reported health outcomes) is not known. The Panel therefore concludes that these studies are not useful as such for setting DRVs for vitamin D, and may only be used to support the outcome of the characterisation of the vitamin D intake-status relationship undertaken by the Panel under conditions of minimal endogenous vitamin D synthesis.

The Panel concludes that a serum 25(OH)D concentration of 50 nmol/L is a suitable target value to set the DRVs for vitamin D, for all age and sex groups (adults, infants, children, pregnant and lactating women). For setting DRVs for vitamin D, the Panel considers the dietary intake of vitamin D necessary to achieve this serum 25(OH)D concentration. As for other nutrients, DRVs for vitamin D are set assuming that intakes of interacting nutrients, such as calcium, are adequate.

EFSA undertook a meta-regression analysis of the relationship between serum 25(OH)D concentration and total vitamin D intake (habitual diet, and fortified foods or supplements using vitamin D_3). Randomised trials conducted in a period of assumed minimal endogenous vitamin D synthesis were identified through a comprehensive literature search and a review undertaken for EFSA by an external contractor. The analysis was performed using summary data from 83 trial arms (35 studies), of which nine were on children (four trials, age range: 2–17 years) and the other arms were on adults (excluding pregnant or lactating women). Data were extracted for each arm of the individual trials. The meta-regression analysis resulted in two predictive equations of achieved serum 25(OH)D concentrations: one derived from an unadjusted model (including only the natural log of the total intake) and one derived from a model including the natural log of the total intake and adjusted for a number of relevant factors (baseline 25(OH)D concentration, latitude, study start year, type of analytical method applied to assess serum 25(OH)D, assessment of compliance) set at their mean values.

The Panel considers that the available evidence does not allow the setting of Average Requirements (ARs) and Population Reference Intakes (PRIs), and therefore defines Adequate Intakes (AIs) instead, for all population groups.

For adults, the Panel sets an AI for vitamin D at 15 µg/day. This is based on the adjusted model of the meta-regression analysis, and considering that, at this intake, most of the adult population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L.

For children aged 1–17 years, the Panel sets an AI for vitamin D for all children at 15 µg/day. This is based on the adjusted model of the meta-regression analysis on all trials (adults and children) as well as on a stratified analysis by age group (adults versus children).

For infants aged 7–11 months, the Panel sets an AI for vitamin D at 10 µg/day, considering four recent trials on the effect of vitamin D supplementation on serum 25(OH)D concentration in (mostly) breastfed infants.

For pregnant and lactating women, the Panel considers that the AI is the same as for non-pregnant non-lactating women, i.e. 15 µg/day.

The Panel underlines that the meta-regression analysis on adults and children was done on data collected under conditions of minimal cutaneous vitamin D synthesis. In the presence of cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may even be zero.

154 **TABLE OF CONTENTS**

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on nutrient and energy intakes for the European Community.[4] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context the EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002,[5] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance the EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;

[4] Scientific Committee for Food, 1993. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food, 31st series. Food – Science and Technique, European Commission, Luxembourg, 248 pp.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;
- Protein;
- Dietary fibre.

Following on from the first part of the task, the EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, the EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

1. Introduction

In 1993, the Scientific Committee for Food (SCF) adopted an opinion on nutrient and energy intakes for the European Community and derived for vitamin D acceptable ranges of intakes for adults, according to the amount of endogenous synthesis of vitamin D (SCF, 1993). Acceptable ranges of intakes were also set for infants aged 6–11 months, and children aged 4–10 and 11–17 years, according to the amount of endogenous vitamin D synthesis, while a single reference value for the age range 1–3 years was selected. The same reference value was proposed for pregnancy and for lactation.

In the present Opinion, vitamin D intake is expressed in µg and concentrations in blood are expressed in nmol/L.[6]

2. Definition/category

2.1. Chemistry

Vitamin D belongs to the fat-soluble vitamins. It is the generic term for ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), which are formed from their respective provitamins ergosterol and 7-dehydrocholesterol (7-DHC) involving ultraviolet-B (UV-B) irradiation, that opens the B-ring of the molecules, and subsequent thermal isomerisation (Figure 1). Vitamin D_2 differs from vitamin D_3 in the side chain where it has a double bond between C22 and C23 and an additional methyl group on C24 (Binkley and Lensmeyer, 2010). The molecular masses of ergocalciferol and cholecalciferol are 396.65 and 384.64 g/mol, respectively. In this assessment, the term vitamin D refers to both vitamin D_3 and vitamin D_2 unless the specific form is indicated.

Analytical methods for the quantification of vitamin D in serum are discussed in Section 2.4.1.

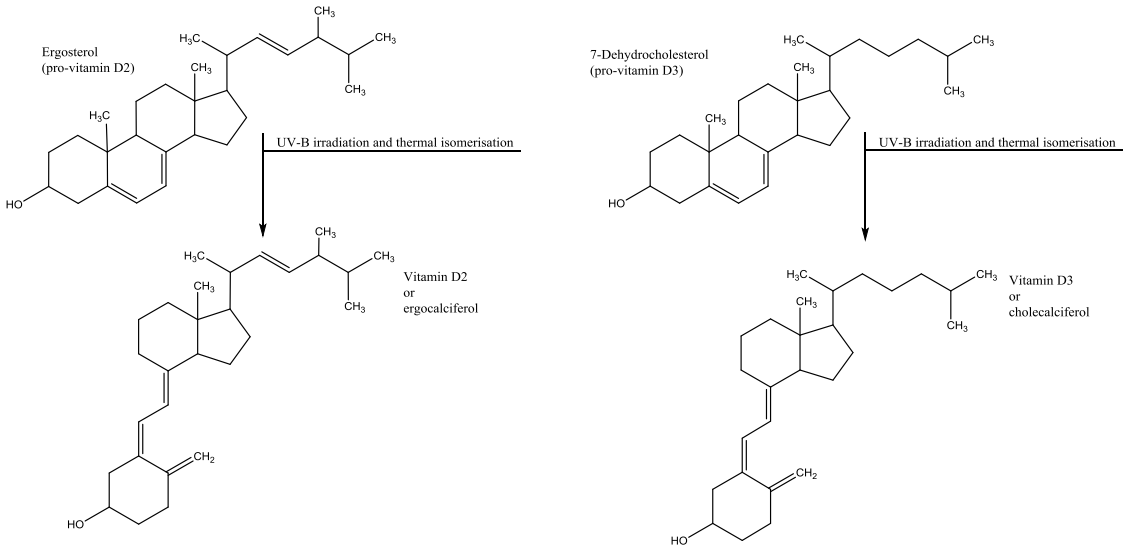

Figure 1: Vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) with their respective provitamins. Based on data from (Norman, 2012).

[6] For conversion between µg and International Units (IU) of vitamin D intake: 1 µg = 40 IU and 0.025 µg = 1 IU. For conversion between nmol/L and ng/mL for serum 25(OH)D concentration: 2.5 nmol/L = 1 ng/mL.

2.2. Function of vitamin D

2.2.1. Biochemical functions

In the body, vitamin D_2 and D_3 are converted to the main circulating form, 25-hydroxyvitamin D ($25(OH)D_2$ or $25(OH)D_3$ termed calcidiols). It can be transformed into the biologically active metabolites 1,25-dihydroxy-ergocalciferol ($1,25(OH)_2D_2$) or 1,25-dihydroxy-cholecalciferol ($1,25(OH)_2D_3$) called calcitriols (Section 2.3.6). The term 25(OH)D refers to both $25(OH)D_2$ and $25(OH)D_3$ and $1,25(OH)_2D$ refers to both $1,25(OH)_2D_3$ and $1,25(OH)_2D_2$ unless the specific form is indicated.

The principal function of the biologically active metabolite $1,25(OH)_2D$ is to maintain calcium and phosphorus homeostasis in the circulation, together with parathyroid hormone (PTH) and fibroblast growth factor (FGF-23) (EFSA NDA Panel, 2012a; Jones, 2013). If the serum ionised calcium concentration falls below a normal concentration of about 1.1–1.4 mmol/L, a cascade of events occurs to restore and maintain it within the range required for normal cellular and tissue functions (Mundy and Guise, 1999; Weaver and Heaney, 2006; Ajibade et al., 2010; EFSA NDA Panel, 2015a). The main target tissues of $1,25(OH)_2D$ are the intestine, kidneys and the bone (Figure 2, Section 2.3.6.). In the intestine, $1,25(OH)_2D$ binds to the vitamin D receptor (VDR) to facilitate calcium and phosphorus absorption by active transport. In the kidneys, $1,25(OH)_2D$ stimulates the tubular reabsorption of calcium dependent on PTH that increases the production of $1,25(OH)_2D$ from 25(OH)D in the proximal tubule (Holt and Wysolmerski, 2011). $1,25(OH)_2D$ also downregulates the activity of the enzyme 1α-hydroxylase (CYP27B1), which is responsible for the conversion of 25(OH)D to $1,25(OH)_2D$ in the kidney. In the bone, PTH and $1,25(OH)_2D$ interact to activate the osteoclasts responsible for bone resorption. Osteoclasts then release hydrochloric acid and hydrolytic enzymes to dissolve the bone matrix and thereby release calcium and phosphorus into the circulation (Holick, 2006a, 2007).

The metabolite $1,25(OH)_2D$ is also important in other tissues (Bouillon et al., 2008; EFSA NDA Panel, 2012a; Jones, 2014) that have VDRs as well as the 1α-hydroxylase to convert 25(OH)D into $1,25(OH)_2D$ (Holick, 2007). For example, the parathyroid cells express the VDR and the 1α-hydroxylase, which allows the local formation of $1,25(OH)_2D$. $1,25(OH)_2D$ suppresses the expression of the gene encoding PTH and among other actions, inhibits proliferation of parathyroid cells (Bienaime et al., 2011) (Figure 2).

Other functions of $1,25(OH)_2D$ include cell differentiation and antiproliferative actions in various cell types, such as bone marrow (osteoclast precursors and lymphocytes), immune cells, skin, breast and prostate epithelial cells, muscle, and intestine (Norman, 2008, 2012; Jones, 2014).

Vitamin D can be characterised as a prohormone, because it requires two steps of activation to become biologically active (Jones, 2013).

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

Clinical symptoms of vitamin D deficiency manifest as rickets in children and osteomalacia in adults (Sections 5.1.1., 5.1.1.1.2., 5.1.1.2.2.). Both are caused by the impaired mineralisation of bone due to an inefficient absorption of dietary calcium and phosphorus, and both are associated with an increase in PTH concentration to prevent hypocalcaemia (Holick, 2006a; Holick et al., 2012).

Rickets is characterised by a triad of clinical symptoms: skeletal changes (with deformities, craniotabes, growth retardation), radiologic changes (widening of the metaphyseal plates, decreased mineralisation, deformities) and increases in bone alkaline phosphatase (ALP) activity in serum (Wharton and Bishop, 2003). Depending on the severity and duration of vitamin D deficiency, initial hypocalcaemia progresses to normocalcaemia and hypophosphatemia, because of increased PTH secretion and, finally to combined hypocalcaemia and hypophosphatemia when calcium can no longer be released from bone. Osteomalacia is characterised by increased bone resorption and suppression of new bone mineralisation (Lips, 2006), and serum calcium concentration is often normal (2.25–2.6 mmol/L) despite the undermineralisation of bone. The clinical symptoms of vitamin D deficiency in adults are less pronounced than in children, and may include diffuse pain in muscles and bone and specific fractures. Muscle pain and weakness (myopathy) that accompany the skeletal symptoms in older adults may contribute to poor physical performance, increased risk of falls/falling and a higher risk of bone fractures.

Prolonged vitamin D insufficiency may lead to low bone mineral density (BMD) and may dispose older subjects, particularly post-menopausal women, for osteoporosis, a situation characterised by a reduction in bone mass, reduced bone quality and an increased risk of bone fracture, predominantly in the forearm, vertebrae, and hip (Heaney et al., 2000; Gaugris et al., 2005; Holick, 2007; Avenell et al., 2014).

2.2.2.2. Excess

Following ingestion of pharmacological doses (e.g. 125–1 000 µg/day) of vitamin D over a period of at least one month, the concentration of serum 25(OH)D increases, while that of $1,25(OH)_2D$ is unchanged or even reduced (EFSA NDA Panel, 2012a; Jones, 2014). High serum 25(OH)D concentrations (> 220 nmol/L) may lead to hypercalcaemia, which may eventually lead to soft tissue calcification and resultant renal and cardiovascular damage (Vieth, 1999; Zittermann and Koerfer, 2008).

In revising the Tolerable Upper Intake Levels (ULs) for vitamin D (EFSA NDA Panel, 2012a), data on possible associations between vitamin D intake or 25(OH)D concentration and adverse long-term health outcomes were considered. However, no studies reported on associations between vitamin D intake and increased risk for adverse long-term health outcomes. Studies reporting on an association between 25(OH)D concentration and all-cause mortality or cancer were inconsistent. For adults, hypercalcaemia was selected as the indicator of hypervitaminosis D or vitamin D toxicity (EFSA NDA Panel, 2012a). Two studies in men supplemented with doses between 234 and 275 µg/day vitamin D_3 showed no association with hypercalcaemia (Barger-Lux et al., 1998; Heaney et al., 2003a), and a No Observed Adverse Effect Level (NOAEL) of 250 µg/day was established (Hathcock et al., 2007). Taking into account uncertainties associated with these two studies, the UL for adults was set at 100 µg/day. Two studies in pregnant and lactating women, both using doses of vitamin D_2 and D_3 up to 100 µg/day for several weeks to months, did not report adverse effects for either mothers or their offspring (Hollis and Wagner, 2004b; Hollis et al., 2011). Thus, the UL of 100 µg/day applies to all adults, including pregnant and lactating women (EFSA NDA Panel, 2012a).

There is a paucity of data on high vitamin D intakes in children and adolescents. Considering phases of rapid bone formation and growth and the unlikelihood that this age group has a lower tolerance for vitamin D compared to adults, the UL was set at 100 µg/day for ages 11–17 years (EFSA NDA Panel, 2012a). The same consideration applied also to children aged 1–10 years, but taking into account their smaller body size, a UL of 50 µg/day was selected (EFSA NDA Panel, 2012a).

For infants, data relating high vitamin D intakes to impaired growth and hypercalcaemia (Jeans and Stearns, 1938; Fomon et al., 1966; Ala-Houhala, 1985; Vervel et al., 1997; Hyppönen et al., 2011)

were used as indicators in the previous risk assessment by the SCF to set the UL at 25 µg/day (SCF, 2002a). The Panel retained the UL of 25 µg/day and noted that no long-term studies were available (EFSA NDA Panel, 2012a).

The Panel notes that two randomised controlled trials (RCTs) have been published after the assessment of the UL by the EFSA NDA Panel (2012a). In both RCTs, infants received vitamin D_3 supplementation of 10, 30 or 40 µg/day, for a period of three months (Holmlund-Suila et al., 2012) or 12 months (Gallo et al., 2013), with concomitant increases in mean serum 25(OH)D concentrations (Section 5.1.1.2.1.). In the shorter term study (Holmlund-Suila et al., 2012), hypercalcaemia or hypercalciuria did not occur at any dose of vitamin D_3 supplemented. In the longer term study (Gallo et al., 2013), the dose of 40 µg/day was discontinued prematurely because of elevated serum 25(OH)D concentrations above 250 nmol/L, a criterion *a priori* chosen by the authors to indicate hypervitaminosis D.

2.3. Physiology and metabolism

2.3.1. Cutaneous synthesis of vitamin D

Vitamin D_3 is synthesised in the skin from 7-DHC following exposure to UV-B irradiation, which, by opening the B-ring, leads to the formation of previtamin D_3 in the upper layers of the skin that, immediately after its formation, thermally isomerises to vitamin D_3 in the lower layers of the skin (Figure 1) (Engelsen et al., 2005; EFSA NDA Panel, 2012a). The synthesis of vitamin D_3 in the skin is a function of the amount of UV-B irradiation reaching the dermis and the availability of 7-DHC and heat. During summer months or following exposure to artificial UV-B irradiation, the synthesis of vitamin D_3 in the skin may be the main source of vitamin D. Dietary intake of vitamin D is essential in case endogenous synthesis, due to insufficient UV-B exposure, is lacking or insufficient. With increasing latitude, both the qualitative and quantitative properties of sunlight are not sufficient in parts of the year for vitamin D_3 synthesis in the skin to take place, leading to the so-called vitamin D winter (Engelsen et al., 2005). For example, in Rome, Italy (41.9°N), the vitamin D winter is from November through February; in Berlin, Germany (52.5°N) or Amsterdam, the Netherlands (52.4°N), it is between October and April (Tsiaras and Weinstock, 2011); and in Tromsø, Norway (69.4°N), it is between beginning of October through mid-March (Engelsen et al., 2005).

Besides considering latitude and season, a UV-index can be used to estimate vitamin D_3 synthesis in the skin (Brouwer-Brolsma et al., 2016) (Section 5.3.2.1.), assuming that sun exposure with a UV-index < 3 does not supply the body with sufficient vitamin D (Webb and Engelsen, 2006; McKenzie et al., 2009). The categorisation of studies where subjects are exposed to a UV-index < 3 and ≥ 3 can be done using data from the World Health Organization (WHO).[7] However, it has been found that, even when the UV-index is < 3, there may be endogenous vitamin D synthesis (Seckmeyer et al., 2013). Another approach to estimate vitamin D_3 synthesis in the skin (Brouwer-Brolsma et al., 2016) is to use a simulation model that estimates the exposure to UV-irradiation at 45°N at any time of the year in the middle of the day, assuming that this may result in vitamin D synthesis in the skin (Webb, 2006; Webb and Engelsen, 2006). For example, at 50°N, it is assumed that there is no appreciable vitamin D synthesis from mid-November till February.

In addition to latitude and season, the vitamin D synthesis in the skin of humans is affected by several other external factors. The ozone layer effectively absorbs UV-B irradiation. Clouds, when completely overcast, can attenuate the UV-B irradiation by as much as 99%. Surface, especially snow, can however reflect up to 95% of the UV-B irradiation. Time spent outdoors, the use of

[7] http://www.who.int/uv/intersunprogramme/activities/uv_index/en/index3.html

sunscreen, and clothing also affect the sun-induced vitamin D synthesis in the skin (Engelsen, 2010).

After adjustment for potential confounders, individuals with initially lower serum 25(OH)D concentration (below 37.5 nmol/L) responded more quickly to UV-B exposure (and thus synthesised vitamin D in the skin) than individuals with higher concentrations (Brustad et al., 2007). The sun-induced vitamin D synthesis can be up to six times higher in subjects with light skin, compared to people with dark skin because of the higher content of melanin in the latter group (Webb and Engelsen, 2006). The ability to vitamin D synthesis in the skin decreases with age (Lamberg-Allardt, 1984; MacLaughlin and Holick, 1985).

UV-B irradiation regulates total synthesis of vitamin D_3 in the skin, as both previtamin D_3 and vitamin D_3 present in the skin are photodegraded to biologically inert isomers following UV-B exposure (Webb et al., 1989). This down-regulation of vitamin D synthesis in the skin prevents vitamin D toxicity due to prolonged sun exposure (Holick, 1994). Vitamin D intoxication by UV-B irradiation has not been reported.

The Panel notes that sun exposure may contribute a considerable and varying amount of vitamin D available to the body. The Panel considers that the association between vitamin D intake and status for the purpose of deriving Dietary Reference Values (DRVs) for vitamin D should be assessed under conditions of minimal endogenous vitamin D synthesis (Section 5.3.2.).

2.3.2. Intestinal absorption

Vitamin D from foods is absorbed throughout the small intestine, mostly in the distal small intestine. Studies using radiolabeled compounds indicate that the absorption efficiency of vitamin D varies between 55 and 99% (mean 78%) in humans, with no discrimination between vitamin D_2 and D_3 (Thompson et al., 1966; Lo et al., 1985; Jones, 2014; Borel et al., 2015; Reboul, 2015).

Due to the fat soluble characteristics of vitamin D, the absorption process is more efficient in the presence of biliary salts and when dietary fat is present in the lumen of the small intestine. A systematic review on a limited number of studies (generally reporting not statistically significant results) suggests that an oil vehicle improves the absorption of vitamin D, as shown by a greater serum 25(OH)D response, compared with a powder or an ethanol vehicle (Grossmann and Tangpricha, 2010). However, few data on the effect of the food matrix on vitamin D absorption (vitamin D_2 or vitamin D_3) have been published and the effect of the supplement matrix is not clear, as reviewed by Borel et al. (2015). A recent study reports that vitamin D_2 when given as supplement was more effective in increasing serum 25(OH)D_2 than vitamin D_2-fortified bread (Itkonen et al., 2016). Data suggest that age *per se* has no effect on vitamin D absorption efficiency (Borel et al., 2015). The vitamin D absorbed from the intestine is incorporated into chylomicrons that reach the systemic circulation through the lymphatic system (Jones, 2013) where it is released from chylomicrons by action of lipoprotein lipase upon arrival in the tissues.

The Panel considers that the average absorption of vitamin D from a usual diet is about 80%, that limited data are available on the effect of the food or supplement matrix on absorption of vitamin D (vitamin D_2 or D_3), and that age per se has no effect on vitamin D absorption efficiency.

2.3.3. Transport in blood

Transport of vitamin D from skin to storage tissue or to the liver is carried out by a specific plasma protein called vitamin D-binding protein (DBP). Transport of vitamin D_2 or D_3 from the diet to storage depots or liver is on chylomicrons, although some evidence indicates that transfer from chylomicrons to DBP occurs. Vitamin D from cutaneous synthesis or dietary sources is taken up

within hours for activation (hydroxylation) in the liver or for storage especially in skeletal muscle and adipose tissue (Jones, 2013).

After hydroxylation of vitamin D in the liver, serum 25(OH)D concentrations in the blood reflect the amount of vitamin D attained from both cutaneous synthesis (Section 2.3.1) and dietary sources (Section 2.3.2). In the blood, 85–90% of 25(OH)D is transported bound to DBP, 10–15% is bound to albumin, and < 1% is free (Bikle et al., 1985; Powe et al., 2013; Chun et al., 2014; Yousefzadeh et al., 2014). In a second hydroxylation step, which takes place mainly in the kidney, but also in other tissues, $1,25(OH)_2D$ may be formed (Section 2.3.6.). In the blood, $1,25(OH)_2D$ is primarily transported bound to DBP and albumin (Bikle et al., 1986; Jones et al., 1998; Powe et al., 2013).

The serum concentration of 25(OH)D is approximately 1 000 times higher than that of $1,25(OH)_2D$. An overview of reported 25(OH)D concentrations from studies in 17 European countries (Spiro and Buttriss, 2014) and other recent European data ((Thiering et al., 2015) in Germany) shows that mean/median concentrations (Section 2.4.1.) range from about 20 to 95 nmol/L in adults or children.

While serum 25(OH)D has a half-life of approximately 13–15 days (Jones KS et al., 2012) (Section 2.4.1) due to its strong affinity for DBP, serum $1,25(OH)_2D$ has a half-life measured in hours (Jones et al., 1998; IOM, 2011).

2.3.4. Distribution to tissues

Within hours of ingestion (Section 2.3.2) or synthesis in the skin (Section 2.3.1), vitamin D is distributed to the liver (Sections 2.3.3. and 2.3.6., Figure 2) or delivered as either vitamin D or its metabolites to the storage tissues, especially skeletal muscle and adipose tissue (Section 2.3.5). The vitamin D from dietary sources is released from the chylomicrons by action of the enzyme lipoprotein lipase upon arrival in the tissues. Serum 25(OH)D and $1,25(OH)_2D$ are released from DBP to various tissues such as bone, intestine, kidney, pancreas, brain and skin. Upon release from DBP, $1,25(OH)_2D$ is bound intracellularly to VDR (Section 2.3.6) (Gropper et al., 2009). 25(OH)D is taken up from the blood into tissues, probably by protein-binding (Mawer et al., 1972).

2.3.5. Storage

The long-term storage sites of vitamin D include mainly the adipose tissue, muscle, liver and other tissues (Heaney et al., 2009; Whiting et al., 2013).

Adipose tissue is a major repository in the body for vitamin D (Blum et al., 2008) and, in subjects with no vitamin D_2 supplementation, vitamin D was found in adipocyte lipid droplets as both vitamin D_3 and its metabolites ($25(OH)D_3$ and $1,25(OH)_2D_3$) (Malmberg et al., 2014).

Studies have consistently reported an inverse relationship between body mass index (BMI)/body fat and serum 25(OH)D concentrations, as reviewed in Vanlint (2013). The mechanisms for this relationship are not fully understood. They have been suggested, among others, to include a 'trapping'/sequestration of vitamin D in the body tissues, particularly in adipose tissue in overweight and obese individuals (Wortsman et al., 2000; Parikh et al., 2004; Blum et al., 2008; Jungert et al., 2012), a volumetric dilution of the vitamin D in obese subjects (Drincic et al., 2012), and altered behaviour of obese subjects resulting in less cutaneous vitamin D synthesis in the skin (Vanlint, 2013).

2.3.6. Metabolism

Activation of vitamin D involves two steps. The first occurs after vitamin D is released from DBP to the liver, where it undergoes 25-hydroxylation to 25(OH)D (Holick, 2006b; IOM, 2011) (Figure 2). Both a mitochondrial enzyme (CYP27A1) and several microsomal enzymes (including CYP2R1, CYP3A4 and CYP2J3) are able to carry out the 25-hydroxylation of vitamin D_2 or vitamin D_3 (Jones et al., 2014). The 25-hydroxylation is more efficient with low serum $1,25(OH)_2D$ concentrations than with 'normal' serum $1,25(OH)_2D$ concentrations (Gropper et al., 2009). The product of the 25-hydroxylation step, 25(OH)D, is bound to DBP (Section 2.3.3) and transported to the kidneys.

The second step is the 1α-hydroxylation of 25(OH)D primarily in the kidney (Jones, 2014). Apart from the kidneys, $1,25(OH)_2D$ is also produced in an autocrine way in other organs such as bone cells and parathyroid cells. The placenta is one of the extrarenal sites for production of $1,25(OH)_2D$ by the 1α-hydroxylase. This local production supports the calcium demand of the fetus and does not contribute to the circulating concentration of $1,25(OH)_2D$ of the mother (Jones, 2014).

The activity of the 1α-hydroxylase (Section 2.2.1.) is regulated by calcium, phosphate, and their regulating hormones (Figure 2). Any interruption of this activation process, due to, for example, liver or kidney disease, may lead to vitamin D deficiency (Section 2.2.2.1) (Holick, 2007). After its production, $1,25(OH)_2D$ is transported bound to DBP in the blood (Section 2.3.3) to the target tissues (Section 2.2.1).

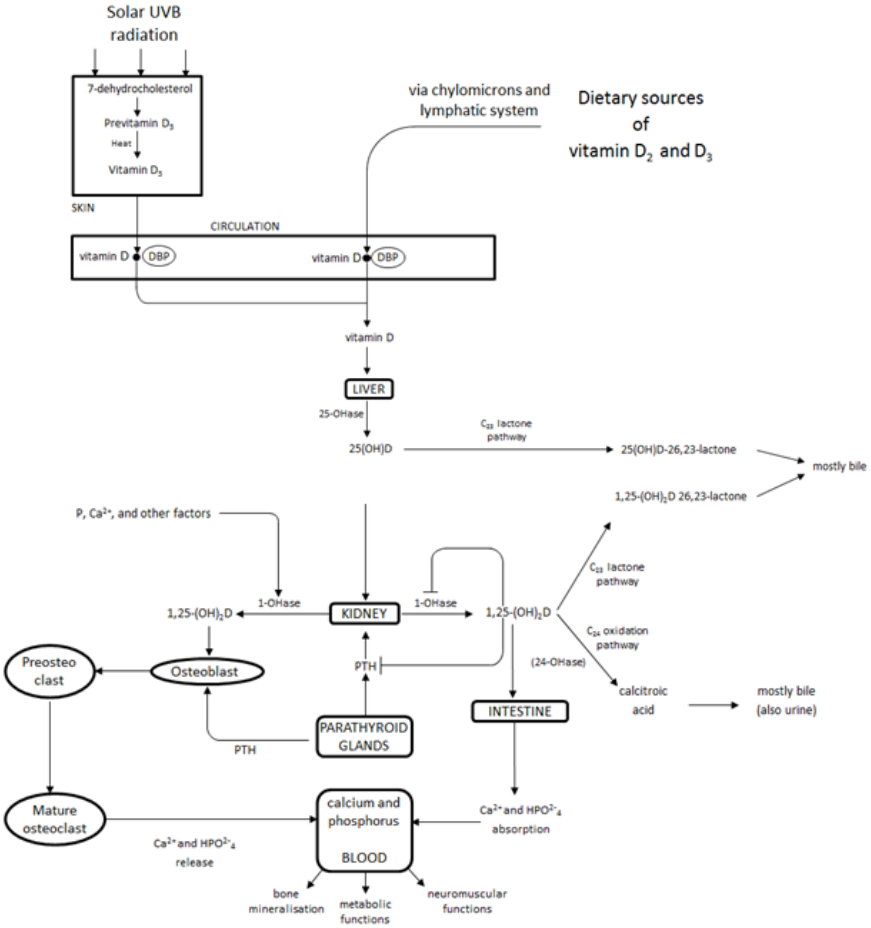

Figure 2: Metabolism of vitamin D. Based on data from Holick (2006a).

The metabolite $1,25(OH)_2D$ is fairly unstable without the attachment to carrier proteins (Lehmann and Meurer 2010; Norman 2008). Once at the target cells, $1,25(OH)_2D$ must be released from the DBP and current evidence suggests that it is the unbound fraction that has access to the target cells (Section 2.4.2.). Free $1,25(OH)_2D$ taken up by target cells is either rapidly metabolised or bound to VDRs (Lehmann and Meurer, 2010). VDRs are involved in various regulatory processes that stand beyond classical homeostasis of calcium and phosphate. VDRs have been identified in the cardiovascular system and most cell types in the immune system, and also in other tissues like pancreas, skeletal muscle, lung, central nervous system, and reproductive system (Holick, 2004; Bischoff-Ferrari, 2010). Thus, $1,25(OH)_2D$ in association with VDR has a biological function not limited to bone, intestine, kidneys and parathyroid glands, but throughout the body, regulating many functions.

Upon binding of $1,25(OH)_2D$, the VDR undergoes conformational changes that will allow interaction with several other transcriptional factors within the nucleus in the target cells (Bouillon et al., 2008). To interact with transcriptional factors and affect gene transcription, the active VDR must form a heterodimer with the retinoid receptor, and this heterodimer can then bind to selector or promoter sites of the target cell DNA. This new complex recruits various activators and co-depressors that affect gene expression. This can include protein synthesis and secretion, cellular proliferation or differentiation. Several factors determine the overall cellular responses, including cell type and cell number, availability of VDR and the affinity of the $1,25(OH)_2D$ to this receptor (Jones et al., 1998).

According to the review by Jones (2013), although vitamin D_2 and D_3 present structural differences (Figure 1, Section 2.1.), qualitatively, they trigger an identical set of biological responses in the body (Figure 2), primarily by the regulation of gene expression mediated by the same VDR. None of the steps in the specific vitamin D signal transduction cascade appears to discriminate between the vitamin D_2 and vitamin D_3 at the molecular level (Jones, 2013). Vitamins D_2 and D_3 are considered biologically equivalent in terms of their ability to cure rickets (Jones, 2013).

Potential differences in the biological potencies of vitamin D_2 and D_3 have been addressed in studies that measured increases in plasma 25(OH)D concentrations (Section 2.4.1.) as a surrogate non-functional marker of biological activity after supplemental vitamin D_2 or D_3 (Jones, 2013; Lehmann et al., 2013; Itkonen et al., 2016). These studies have consistently shown that administration of vitamin D_2 supplements decreases the percentage contribution of vitamin D_3 to the total pool of vitamin D undergoing 25-hydroxylation, and that this decrease is accompanied by a fall in absolute serum $25(OH)D_3$ concentrations. Data from toxicity and repletion studies suggest some preferential non-specific catabolism of vitamin D_2, accelerating its destruction (Jones, 2013). Data also suggest that vitamin D_3 may be the preferred substrate for hepatic 25-hydroxylation (Holmberg et al., 1986; Tripkovic et al., 2012). A meta-analysis comparing supplementation studies with vitamin D_2 and D_3 concluded that, even though bolus doses of vitamin D_3 (> 125 µg/day) were more efficacious for raising total serum 25(OH)D concentration compared with vitamin D_2 doses, the differences between the two forms of vitamin D supplements disappeared when given as lower daily doses (Tripkovic et al., 2012).

The catabolism of 25(OH)D and $1,25(OH)_2D$ in the body involves inactivation by 24-hydroxylation, which gives rise initially to $24,25(OH)_2D$ (preventing the activation of 25(OH)D to $1,25(OH)_2D$ (Jones G et al., 2012; Biancuzzo et al., 2013)) and to $1,24,25(OH)_3D$ (i.e. 1,24,25-trihydroxyvitamin D, then leading to calcitroic acid) (Section 2.3.7.). Following vitamin D supplementation, 24-hydroxylase (CYP27A1) is upregulated with a lag of several weeks (Wagner et al., 2011).

There is some evidence that certain products of the degradation pathway are functional. For example, the $24,25(OH)_2D_3$ is of importance in bone mineralisation and PTH suppression (Jones, 2014). Others have indicated that the 24-hydroxylated metabolites are important in fracture repair,

623 although the vast majority of the evidence points towards 24-hydroxylation being a step in the
624 pathway of inactivation (Jones, 2014).

625 The Panel notes that $1,25(OH)_2D$ in association with VDR has a biological function not limited to
626 bone, intestine, kidneys and parathyroid glands, but throughout the body, regulating many functions.
627 The Panel also notes the conflicting results regarding the potential differences in the biological
628 potencies and catabolism of vitamin D_2 and D_3. The Panel thus considers that the association
629 between vitamin D intake and status for the purpose of deriving DRVs for vitamin D, may need to
630 be investigated considering vitamin D_2 and D_3 separately (Section 5.3.2.).

631 **2.3.7. Elimination**

632 There are two main pathways of degradation, the C23 lactone pathway, and the C24 oxidation
633 pathway (Section 2.3.6. and Figure 2) (Holick, 1999; Jones, 2014). Vitamin D metabolites in the
634 body are degraded in an oxidative pathway involving stepwise side-chain modifications by the
635 actions of CYP24A1 (24-hydroxylase). $1,25(OH)_2D$ is a strong controller of its own degradation by
636 stimulating the 24-hydroxylase (IOM, 2011). After several steps, one of the final product of the C24
637 oxidation pathway, i.e. calcitroic acid, is excreted, mainly in the bile and thus in the faeces. Human
638 CYP24A1 also catalyses, though to a lesser extent, the 23-hydroxylation of both 25(OH)D and
639 $1,25(OH)_2D$ leading, in sequential steps, to 25(OH)D-26,23-lactone and $1,25(OH)_2D$-26,23-lactone,
640 respectively (Jones et al., 2014). $1,25(OH)_2D$ can also be epimerised by the conversion of the
641 configuration of the hydroxyl-group at the C-3 of the A ring to 3-epi-1α,25$(OH)_2D$. Other vitamin D
642 metabolites can be epimerised as well and are then less biologically active. 3-epi-1α,25$(OH)_2D$
643 showed some transcriptional activity toward target genes and induction of anti-
644 proliferative/differentiation activity in human leukaemia cells (Kamao et al., 2004).

645 2.3.7.1. Faeces and urine

646 The majority (around 70%) of the metabolites of the vitamin D pathways of degradation are
647 excreted in the bile (Jones, 2014). Due to active renal re-uptake, the urinary excretion of vitamin D
648 metabolites is low.

649 The Panel notes that the main route of excretion of vitamin D metabolites is via the faeces.

650 2.3.7.2. Breast milk

651 Breast milk accounts for a small part of the vitamin D elimination in lactating women (Taylor et al.,
652 2013). The concentration of vitamin D in breast milk is higher than that of 25(OH)D (and of
653 $1,25(OH)_2D$), and vitamin D passes more readily from the circulation into the breast milk than
654 25(OH)D (Makin et al., 1983; Hollis et al., 1986). In general, mean vitamin D concentrations in
655 breast milk of healthy lactating women, unsupplemented or supplemented with vitamin D below the
656 UL, are low and in the range of 0.25–2.0 µg/L (Dawodu and Tsang, 2012; EFSA NDA Panel,
657 2013). There is a general agreement that human milk does not contain sufficient vitamin D to
658 prevent rickets in the breast-fed infant (Olafsdottir et al., 2001).

659 The amount of vitamin D in human milk modestly correlates with maternal vitamin D intake up to
660 about 18 µg/day, with evidence for a lower response in African-American compared to Caucasian
661 women (who had mean maternal serum 25(OH)D concentration of about 67 and 112 nmol/L,
662 respectively) (Specker et al., 1985; EFSA NDA Panel, 2012a).

663 Vitamin D supplementation starting in late pregnancy (i.e. after 27 weeks of gestation) (Wall et al.,
664 2015) or early lactation (Ala-Houhala et al., 1988a; Hollis and Wagner, 2004b) may increase the
665 vitamin D concentration of breast milk, though only modestly unless high supplemental doses are

used. For example, Hollis and Wagner (2004b) supplemented 18 lactating mothers within one month after birth with 10 µg vitamin D_3 and with either 40 µg or 90 µg vitamin D_2 daily for three months. Mean serum total 25(OH)D concentration increased compared to baseline in both groups (from about 69 to about 90 nmol/L, and from about 82 to about 111 nmol/L, respectively). Mean milk antirachitic activity[8] increased from 35.5 to 69.7 IU/L in the group receiving 50 µg vitamin D/day and from 40.4 to 134.6 IU/L in the group receiving 100 µg vitamin D/day. This was attributable to increases in milk concentrations of both vitamin D and 25(OH)D.

Considering a mean milk transfer of 0.8 L/day during the first six months of lactation in exclusively breastfeeding women (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009), and a concentration of vitamin D in mature human milk of 1.1 µg/L (mid-point of the range of means of 0.25–2.0 µg/L), the secretion of vitamin D into milk during lactation is around 0.9 µg/day.

The Panel considers that secretion of vitamin D into breast milk during the first six months of exclusive breastfeeding is about 0.9 µg/day.

2.3.8. Metabolic links with other nutrients

Vitamin D interacts with other nutrients from the diet. There is interaction between 1,25$(OH)_2$D, calcium and phosphorus that affects mineral and vitamin D metabolism (EFSA NDA Panel, 2015a, 2015c). Administration of potassium salts may alter renal synthesis of 1,25$(OH)_2$-vitamin D (Sebastian et al., 1990; Lemann et al., 1991). Vitamin A has been suggested to interfere with the action of vitamin D. The active metabolite of vitamin A, i.e. retinoic acid, and 1,25$(OH)_2$D regulate gene expression through nuclear receptors (Section 2.3.6.). Data on interactions between vitamin A and vitamin D have been reviewed (SCF, 2002b; EFSA NDA Panel, 2015b). Both 1,25$(OH)_2$D and vitamin K are needed for the synthesis of osteocalcin in the osteoblasts and 1,25$(OH)_2$D regulates the expression of osteocalcin.

2.4. Biomarkers

2.4.1. Plasma/serum concentration of 25(OH)D

Plasma or serum concentration of 25(OH)D represents total vitamin D from exposure to both UV-irradiation (cutaneous synthesis) and dietary sources (Section 2.3.3) and can be used as a biomarker of vitamin D intake in people with low exposure to UV-B irradiation from sunlight (EFSA NDA Panel, 2012a). Serum 25(OH)D has a long half-life of approximately 13–15 days (IOM, 2011; Jones KS et al., 2012) (Section 2.3.3) and is considered a useful marker of vitamin D status (both D_2 and D_3) (Seamans and Cashman, 2009; EFSA NDA Panel, 2012a).

Plasma/serum 25$(OH)D_2$ is of dietary origin only, while plasma/serum 25$(OH)D_3$ may be of dietary or dermal origin (Section 2.3.1.). Body composition has an impact on serum 25(OH)D concentration and an inverse correlation between serum 25(OH)D concentrations and BMI has been observed (Section 2.3.5) (Saneei et al., 2013). Increasing oral vitamin D intake increases 25(OH)D concentration until a plateau is reached after about six weeks, which indicates an equilibrium between the production and degradation of serum 25(OH)D (Vieth, 1999; Viljakainen et al., 2006a).

A linear relationship was reported between vitamin D intake and serum 25(OH)D concentrations up to a total vitamin D intake of 35 µg/day (Cashman et al., 2011a) and 50 µg/day (Cranney et al., 2007). The US Institute of Medicine (IOM, 2011) found a steeper rise in the serum 25(OH)D

[8] Vitamin D antirachitic activity in milk was assessed through measurement of vitamin D_2, vitamin D_3, 25$(OH)D_2$, and 25$(OH)D_3$ concentrations in the milk and conversion of findings into biological activity values with reference data from biological activity assays.

concentrations with vitamin D intakes up to 25 µg/day and a slower, more flattened response when 25 µg/day or more were consumed (Section 5.3.2).

There is an ongoing debate about the optimal range of serum 25(OH)D concentration and the cut-off values for defining deficiency, insufficiency and sufficiency (Jones, 2014) (Section 4). A serum 25(OH)D concentration of 25–30 nmol/L has been proposed as a value below which the risk of rickets and osteomalacia increases (Cashman et al., 2011b). Other health outcomes may also be considered (Sections 4 and 5.1.).

There are numerous methods for the measurement of 25(OH)D in serum (Wallace et al., 2010; Carter, 2011) including high-performance liquid chromatography with UV-detection (HPLC/UV), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and immunoassays (radioimmunoassays RIA, competitive protein binding assays CPBA, enzyme-linked immunosorbent assays ELISA) that are either manual or automated. LC-MS/MS and HPLC methods are considered the gold standard methods (Wallace et al., 2010; Carter, 2011). These methods have the advantage that they can measure 25(OH)D_3 and 25(OH)D_2 separately, which is needed in specific situations (Tai et al., 2010; Carter, 2011). Also, some methods allow detection of other vitamin D metabolites, such as 24,25$(OH)_2$D (Wallace et al., 2010; Carter, 2012). All methods suffered earlier from the lack of a common standard that yielded diverse results.

The Vitamin D External Quality Assessment Scheme (DEQAS) (DEQAS, online) has revealed considerable differences between methods (both within and between laboratories), raising concerns about the comparability and accuracy of different assays and laboratories (Snellman et al., 2010; Carter, 2011; Farrell et al., 2012; Heijboer et al., 2012). The introduction of a standard reference material for vitamin D in human serum by the US National Institute of Standards and Technology (NIST) (NIST, online) has been a step forward in providing a reference measurement procedure (RMP) against which assays could be standardised (Carter, 2012). The Vitamin D Standardization Program (VDSP)[9] has developed protocols for standardising procedures of 25(OH)D measurement in National Health/Nutrition Surveys to promote 25(OH)D measurements that are accurate and comparable over time, location, and laboratory to improve public health practice (Cashman et al., 2013). The VDSP RMP has been joined by a number of commercial methods and laboratories and thus, their results are comparable to LC-MS/MS as regards 25(OH)D concentrations. In the VDSP, LC-MS/MS is the reference method. According to a reanalysis of serum 25(OH)D concentrations using the VDSP protocol, the range of mean concentrations (Section 2.3.3.) in 14 European studies in children and adult populations (including one study in migrants in Finland) was 38.3-65 nmol/L (versus 44.8–69 nmol/L in the originally analysed serum 25(OH)D data) (Cashman et al., 2016).

Thus, there is a range of methodologies available for the measurement of 25(OH)D, and each method has its advantages and limitations (Wallace et al., 2010). Given the lack of consensus on optimal range of serum 25(OH)D concentration and the cut-off values for defining deficiency, insufficiency and sufficiency mentioned above, the Panel considered relevant studies on the relationship between serum 25(OH)D concentration and health outcomes (Section 5.1.), and this review was undertaken irrespective of the analytical method applied to measure serum 25(OH)D concentration. However, analytical methods are considered by the Panel in a sensitivity analysis for the assessment of the relationship between total vitamin D intake and serum 25(OH)D concentration (Section 5.3.2., Appendices C and D).

The Panel considers that serum 25(OH)D concentration can be used as biomarker of vitamin D intake in a population with low exposure to UV-B irradiation (from sunlight, Section 2.3.1.), and of vitamin D status at population level.

[9] https://ods.od.nih.gov/Research/vdsp.aspx

2.4.2. Free serum 25(OH)D concentration

Free serum 25(OH)D is the fraction of serum 25(OH)D (Section 2.3.3) that circulates without being bound to DBP and albumin. This free form accounts for less than 1% of total 25(OH)D in the body, but has been hypothesized to be a potential marker of vitamin D status, because this free fraction is readily available to target cells (Powe et al., 2013; Chun et al., 2014; Johnsen et al., 2014).

The Panel considers that, at present, free serum 25(OH)D concentration cannot be used as biomarker of vitamin D intake and status and that more research is needed to establish the potential of free serum 25(OH)D concentration as a biomarker of vitamin D status.

2.4.3. Plasma/serum 1,25(OH)$_2$D concentration

The biologically active 1,25(OH)$_2$D has a half-life measured in hours (Section 2.3.3.) and is closely linked with blood calcium, PTH, and phosphate concentrations (Sections 2.2.1 and 2.3.6., Figure 2). Zerwekh (2008) considered that plasma/serum 1,25(OH)$_2$D concentration cannot be used to assess vitamin D status, in view of its short half-life and the tight regulation of its concentration. Serum 1,25(OH)$_2$D concentrations do not change according to month of the year (apart in October compared to April) within serum 25(OH)D$_3$ concentrations of 40 nmol/L and 78 nmol/L in healthy children and adults (18 months–35 years) (Chesney et al., 1981). In a cross-sectional study of postmenopausal women, serum 1,25(OH)$_2$D concentration was found to be negatively correlated with serum 25(OH)D concentration at 25(OH)D concentrations $\leq$ 40 nmol/L and positively at concentrations > 40 nmol/L, illustrating a non-linear association between concentrations of serum 25(OH)D and of the active metabolite 1,25(OH)$_2$D (Need et al., 2000). In this study, at serum 25(OH)D concentrations $\leq$ 40 nmol/L (compared to higher concentrations), 1,25(OH)$_2$D concentration was found to be closely related to PTH concentration.

In another study of vitamin D metabolites and calcium absorption in older patients with 25(OH)D concentration < 40 nmol/L (Need et al., 2008), serum 1,25(OH)$_2$D concentrations were significantly decreased concurrent with increases in serum PTH, ALP, and urine hydroxyproline in subjects with serum 25(OH)D < 10 nmol/L. This suggests that this level of substrate is insufficient to maintain serum 1,25(OH)$_2$D concentration, despite secondary hyperparathyroidism.

The Panel considers that, because of the tight homeostatic regulation of 1,25(OH)$_2$D concentration in blood, this marker cannot be used as a biomarker of vitamin D status, but rather reflects vitamin D function.

2.4.4. Serum parathyroid hormone (PTH) concentration

Serum PTH concentration and its relationship with 25(OH)D concentration (via its relationship with 1,25(OH)$_2$D, Sections 2.2.1., 2.3.6. and 2.4.3., Figure 2) has been suggested as a possible biomarker or functional endpoint of vitamin D status. Sai et al. (2011) reviewed 70 studies undertaken in children or adults and showed that it was not possible to set a cut-off value for 25(OH)D concentration using PTH as a reference, due to the low consistency in the cut-off value observed in these studies. A systematic review and meta-analysis of 36 RCTs and four before-after studies that investigated vitamin D supplementation in healthy subjects and the response of 25(OH)D, PTH, BMD, bone markers and calcium absorption, revealed large heterogeneity across the results when comparing 18 RCTs using PTH as a biomarker of vitamin D status (Seamans and Cashman, 2009). In this publication, subgrouping by addition of calcium supplementation or no calcium supplementation suggested an effect of vitamin D supplementation on circulating PTH in the absence of calcium, without important heterogeneity, but not in the presence of calcium supplementation, with strong heterogeneity.

The Panel considers that serum PTH concentration is not a biomarker of vitamin D intake, as serum PTH is also influenced by e.g. serum calcium and phosphate concentrations and other factors. The Panel also considers that PTH concentration in healthy subjects is not a useful biomarker for vitamin D status as assessed by serum 25(OH)D concentration.

2.4.5. Other biomarkers

Since vitamin D is a well-established nutrient in relation to bone, markers of bone formation and turnover (osteocalcin, bone specific ALP and urine N-telopeptide crosslinks) have been considered as markers of long-term status of vitamin D (Bonjour et al., 2014). Low urinary calcium excretion and an increased bone specific ALP activity have been used as biomarkers in the diagnosis of vitamin D deficiency (Section 2.2.2.1.).

Serum concentrations of calcium and inorganic phosphorus that may be low and high PTH serum concentration can help in the diagnosis of rickets or osteomalacia (Section 2.2.2.1.). Structural bone markers (low BMD, rickets or osteoporosis) have also been used as biomarkers of vitamin D status, but have the disadvantage of a slow reaction time, which means that when the condition is diagnosed, bone health may be irreversibly damaged.

The Panel considers that more research is needed to establish the relationship between responses of bone markers (e.g. osteocalcin, bone ALP and urine N-telopeptide crosslinks) to changes in vitamin D status.

2.4.6. Conclusions on biomarkers

The Panel considers that serum 25(OH)D concentration can be used as biomarker of vitamin D intake in a population with low exposure to UV-B irradiation (from sunlight, Section 2.3.1.), and of vitamin D status at population level. The Panel notes that, due to the high variability in 25(OH)D measurements obtained with different analytical methods (Section 2.4.1.), comparison of results from different studies as well as to reference range values has to be done with caution.

2.5. Effects of genotypes

Some polymorphisms of genes encoding proteins involved in vitamin D synthesis, transport and metabolism influence serum 25(OH)D concentrations (Berry and Hypponen, 2011). Two genome-wide association studies (GWAS) (Ahn et al., 2010; Wang et al., 2010), conducted as meta-analyses of data from subjects of European ancestry, identified variants in the genes *DHCR7*, *CYP2R1*, *GC* (group specific component gene) and *CYP24A1*, expressing 7-dehydrocholesterol reductase (DHCR7), 25-hydroxylase, DBP and 24-hydroxylase, respectively.

Mutations in *DHCR7*, going along with an impaired activity of the gene, are seen in the rare Smith-Lemli-Opitz syndrome and result in an accumulation of 7-DHC (Figure 1, Sections 2.1. and 2.3.1.), the substrate for the 25(OH)D synthesis in the skin (Berry and Hypponen, 2011). It has been reported that *DHCR7* mutations are related to a higher vitamin D status and that allele frequencies of *DHCR7* single nucleotide polymorphisms (SNPs) are high at Northern latitudes (0.72 in Europe, 0.41 in Northeast Asia) (Kuan et al., 2013). *CYP2R1* encodes the enzyme primarily responsible for the hydroxylation of vitamin D to 25(OH)D in the liver (Section 2.3.6) and *GC* encodes the DBP that is the major carrier protein for vitamin D and its metabolites (Section 2.3.3). Variants in both genes have been associated with lower 25(OH)D serum concentrations in carriers as compared to non-carriers (Nissen et al., 2014). However, genetic variations in the *GC* gene were also associated with enhanced albumin-bound and free, and therefore readily bioavailable, serum 25(OH)D concentrations (Sections 2.3.3 and 2.4.2.) (Powe et al., 2013; Chun et al., 2014; Johnsen et al., 2014). Season, dietary and supplemental intake may modify the effects on serum 25(OH)D

concentration of the variants in the genes *GC* and *CYP2R1* (Engelman et al., 2013; Waterhouse et al., 2014).

CYP24A1 catalyses the conversion of both 25(OH)D_3 and 1,25$(OH)_2D_3$ into 24-hydroxylated products to be excreted (Sections 2.3.6 and 2.3.7). The reaction is important in the regulation of the concentration of the active 1,25$(OH)_2$D in the kidney and in other tissues (Jones G et al., 2012). Inactivating mutations in the gene encoding this enzyme can cause idiopathic infantile hypercalcaemia (Dinour et al., 2013) and have been linked to other hypercalcaemic conditions causing nephrolithiasis and nephrocalcinosis (Jones G et al., 2012). The possibility that increased expression of *CYP24A1* may be an underlying cause of vitamin D deficiency and progression of disease states has been discussed (Jones G et al., 2012). Associations of the *CYP27B1* genotypes, that code for 1α-hydroxylase (Sections 2.2.1. and 2.3.6.), with 25(OH)D concentrations have also been reported (Hypponen et al., 2009; Signorello et al., 2011) but were not found significant in other studies (Berry and Hyppönen, 2011). With regard to variants of the gene encoding VDR, there is no consistent finding on its relation to serum 25(OH)D concentrations, with the exception of some studies investigating the *Fok-1* polymorphism of VDR although it is not clear how this SNP influences 25(OH)D concentrations (McGrath et al., 2010; Nieves et al., 2012).

The Panel considers that data on the effect of genotypes on vitamin D metabolism are insufficient to be used for deriving the requirements for vitamin D according to genotype variants.

3. Dietary sources and intake data

The major food sources for naturally occurring vitamin D_3 include animal foods such as fatty fish, liver, meat and meat products (particularly offal), and egg yolks (Anses/CIQUAL, 2012; Schmid and Walther, 2013).

Fish (and especially fatty fish and fish liver) have the highest natural content of vitamin D (Schmid and Walther, 2013), presumably derived from an accumulation in the food chain originating from microalgae that contain both vitamin D_3 and provitamin D_3 (Japelt and Jakobsen, 2013). Egg yolk also has a high vitamin D_3 content (Schmid and Walther, 2013), which strongly correlates with the content of vitamin D_3 of the hen's feed (Mattila et al., 1993; Mattila et al., 1999). Animal studies showed that vitamin D_3 and 25(OH)D_3 were effectively transferred from the hen to the egg yolk, depending on the hen's diet (Mattila et al., 2011) and UV-B exposure (Kuhn et al., 2015). The content of vitamin D of meat products varies and depends, among other things, on the contents of vitamin D in the fodder, the fat content of the meat product, and latitude where the animals have grazed (Mattila et al., 2011; Liu et al., 2013).

The vitamin D metabolite 25(OH)D is present in some foods of animal origin in varying amounts (Mattila et al., 1993; Mattila et al., 1995; Mattila et al., 1999; Clausen et al., 2003; Ovesen et al., 2003; Jakobsen and Saxholt, 2009; Cashman, 2012). Due to the suggested higher biological activity of 25(OH)D in foods compared with the native vitamin D, a conversion factor of 5 has been used for 25(OH)D_3 in the calculation of total vitamin D_3 in some food composition tables, including those in the UK, Denmark and Switzerland (Cashman, 2012; Cashman et al., 2012).

Some higher fungi, such as mushrooms, are a natural source of vitamin D_2. Vitamin D_2 is produced in fungi and yeasts by UV-B exposure of provitamin D_2 and the content depends on the amount of UV-B light exposure and time of exposure (Kristensen et al., 2012; Tangpricha, 2012).

Further sources of dietary vitamin D are fortified foods (most often milk, margarine and/or butter, and breakfast cereals) and dietary supplements. Currently, cholecalciferol (vitamin D_3) and

ergocalciferol (vitamin D_2) may be added to both foods[10] and food supplements.[11] The vitamin D content of infant and follow-on formulae and of processed cereal-based foods and baby foods for infants and children is regulated[12].

The stability of vitamin D_3 and 25(OH)D_3 and vitamin D_2 in foodstuffs during cooking has been shown to vary widely with heating process and foodstuffs, with reported retentions in eggs, margarine and bread after boiling, frying and baking of between 40 and 88% (Jakobsen and Knuthsen, 2014).

Published dietary intake data (mean/median and high percentiles) have been collected for adults in 14 European countries and for infants and children in 11 European countries (EFSA NDA Panel, 2012a). Mean intakes of vitamin D in European countries varied according to sex, age and supplementation habits. A direct comparison between countries was difficult as there was a large diversity in the methodology used for dietary assessment, age classification was not uniform, and data from food composition tables used for nutrient intake estimation were different. In the data collected from the different surveys/studies considered, mean/median intake of vitamin D from foods varied from 1.1 to 8.2 µg/day in adults. It varied from 1.7 to 5.6 µg/day in children aged about 1–5 years old, from 1.4 to 2.7 µg/day in children aged about 4–13 years old, and from 1.6 to 4.0 µg/day in children aged about 11–18 years old. When foods and supplements were considered together, mean vitamin D intake varied from 3.1 to 23.5 µg/day in adults. It varied from 8.9 to 12.5 µg/day in infants, from 2.3 µg/day to 9.0 µg/day in children aged about 1.5–3 years old, and from 1.8 µg/day to 6.6 µg/day in children aged about 4–11 years old. In high consumers (95th percentile) in adults, intake was up to 16 µg/day from foods and up to about 24 µg/day from foods and supplements. In high consumers (90th or 95th percentile according to surveys) in infants, children and adolescents, intake from foods and supplements was, respectively, up to 19 µg/day, 15 µg/day and 8 µg/day (EFSA NDA Panel, 2012a).

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

The German-speaking countries (D-A-CH, 2015a) considered a review (Linseisen et al., 2011) following the guidelines of the German Nutrition Society on evidence-based nutrition. A serum 25(OH)D concentration of at least 50 nmol/L was considered advisable for bone health in younger adults (aged less than 65 years), as well as in older adults (65 years and over) (Dawson-Hughes et al., 2005; Linseisen et al., 2011). For younger adults, D-A-CH reported on IOM (2011) and an Irish study undertaken in winter at latitudes comparable with those of Germany (Cashman et al., 2008), that showed that 10 or 20 µg/day of supplemental vitamin D allowed, respectively, 50% or 90–95% of the population to reach a serum 25(OH)D concentration above 50 nmol/L. For older adults, the main focus was the minimisation of the age-related loss of bone mass, the risk of bone fractures, skeletal muscle function and the related risks of loss of strength/mobility/balance, of falls and of fractures (Pfeifer et al., 2000; Bischoff et al., 2003; Pfeifer et al., 2009; Dawson-Hughes et al., 2010; EFSA NDA Panel, 2011; IOM, 2011; Linseisen et al., 2011). D-A-CH considered that studies in older adults supported a protective effect of 10–20 µg/day supplemental vitamin D on loss of the ability to move, on falls, fractures and premature death (Autier and Gandini, 2007; Bischoff-Ferrari et al., 2009a; Bischoff-Ferrari et al., 2009b; LaCroix et al., 2009; Bjelakovic et al., 2011; Linseisen

[10] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26

[11] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

[12] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p.1. and Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 06.12.2006, p. 16-35.

et al., 2011). With 50 µg/day vitamin D, about 90–95% of older adults had a serum 25(OH)D concentration above 50 nmol/L and 50% had a concentration of 75 nmol/L (Cashman et al., 2009). D-A-CH set the Adequate Intake (AI) for all adults at 20 µg/day in situations in which endogenous vitamin D synthesis is absent. D-A-CH considered vitamin D supplements and/or endogenous synthesis to cover the difference between the 'usual' intake (2–4 µg/day) and this value.

The Nordic Council of Ministers (2014)[13] considered a systematic review on vitamin D intake/status and health outcomes (Lamberg-Allardt et al., 2013) (Section 5.1.), based on which a serum 25(OH)D concentration of 50 nmol/L was considered as indicative of a sufficient vitamin D status in adults. They also reported on a systematic review of intervention studies on vitamin D supplementation (Cashman et al., 2011b), from which five studies (Ala-Houhala et al., 1988b; Barnes et al., 2006; Cashman et al., 2008; Viljakainen et al., 2009; Cashman et al., 2011a) were used for specific meta-regression analyses (Section 5.3.1.). Based on two meta-regression analyses in different age groups (Section 5.3.1.), the Average Requirement (AR) for all adults and the Recommended Intake (RI) for adults aged less than 75 years were set at 7.5 and 10 µg/day respectively, assuming some contribution of endogenous synthesis of vitamin D during outdoor activities in summer. An RI was set at 20 µg/day for people with little or no sun exposure during the summer as well as for adults aged 75 years and over, to account for their more limited endogenous synthesis and in consideration of the available data on total mortality, bone health, fractures and falls. A lower intake level of 2.5 µg/day was also set.

The Health Council of the Netherlands (2012) considered that diet provides one third of the vitamin D requirement and sufficient sun exposure provides the remainder. The Council considered that an intake of 11–15 µg/day would be sufficient to reach a serum 25(OH)D concentration > 30 nmol/L for men (18–70 years) and women (18–50 years), derived from data on prevention of rickets in young children. As there was no sign that vitamin D supplementation is required in these groups, the Council rounded the AI down to 10 µg/day. Adults with fair skin and insufficient sun exposure, or with dark skin, or women aged 50–70 years regardless of skin colour and amount of time spent outdoors, were advised to take a vitamin D supplement of 10 µg/day. In older adults (≥ 70 years), an intake of 20–25 µg/day was considered sufficient to reach a 25(OH)D concentration of 50 nmol/L, which was considered advisable for protection against bone fractures (Health Council of the Netherlands, 2000; Cranney et al., 2007; Chung et al., 2009; IOM, 2011). Considering age-related physiological changes (IOM, 2011), for older adults (70 years and over), an Estimated Average Requirement (EAR) and a Recommended Dietary Allowance (RDA) of 10 and 20 µg/day were set. As sun exposure and dietary intake of vitamin D vary in this age group, all older adults were advised to take a vitamin D supplement of 20 µg/day.

IOM (2011) (Appendix B) underlined the interactions between calcium and vitamin D with regard to bone health and the lack of a dose-response relationship between vitamin D intake and bone health. However, based on systematic reviews (Cranney et al., 2007; Chung et al., 2009) and other data published afterwards, IOM considered that total vitamin D intake can be related to change in serum 25(OH)D concentrations under minimal sun exposure and that a dose-response curve for serum 25(OH)D and bone health outcomes can be established. It was considered that serum 25(OH)D concentrations below 30 nmol/L were associated with an increased risk of rickets, impaired fractional calcium absorption and decreased bone mineral content (BMC), in children and adolescents. Concentrations below 30 nmol/L were also associated with an increased risk of osteomalacia and impaired fetal skeletal outcomes, impaired fractional calcium absorption and increased risk of osteomalacia in young and middle-aged adults, and impaired fractional calcium absorption and fracture risk in older adults (IOM, 2011). The IOM considered serum 25(OH)D concentrations > 50 nmol/L as adequate for good bone health for practically all individuals. From the dose-response curve for serum 25(OH)D and bone health outcomes, assuming a normal distribution of requirements, the IOM selected serum 25(OH)D concentrations of 50 nmol/L,

[13] Further abbreviated into NCM in tables.

40 nmol/L and 30 nmol/L as, respectively, the 'RDA[14]-type' and 'EAR[15]-type' reference values, and the 'lower end of the requirement range'. The IOM undertook specific meta-regression analyses (Section 5.3.1.). From the lack of effect of age in these analyses, the IOM concluded that the intake to achieve the EAR-type value of 40 nmol/L was the same across all populations considered. From these analyses, an intake of 10 and 15 µg/day vitamin D would predict a mean serum 25(OH)D concentration higher than the EAR and RDA-type values in children and adults, but given the uncertainties of the analyses, these intakes were selected for the EAR (all adults) and the RDA (until the age of 70 years). For ages 51–70 years, the IOM found no basis to set a specific RDA, as women of this age may have some degree of bone loss but a lower fracture risk than later in life, and as there was generally no effect of vitamin D alone on bone health in this age group. Given the diversity of adults older than 70 years, and uncertainties and variabilities in the physiology of ageing, IOM set the RDA at 20 µg/day, considering the reported significant effect of 2.5 mg of vitamin D every four months (equivalent to 20 µg/day) on the relative risk of fracture in (mainly) men (without calcium supplementation) (Trivedi et al., 2003).

WHO/FAO (2004) considered that a serum 25(OH)D concentration above 27 nmol/L ensures normal bone health. WHO/FAO (2004) reported on the previous approach of IOM (1997) and calculated the recommended nutrient intakes by doubling the vitamin D dietary intake (rounded to the nearest 1.25 µg) required to maintain 25(OH)D concentrations above 27 nmol/L, in order to cover the needs of all individuals irrespective of sunlight exposure. Between 42°N and 42°S, the most efficient way to acquire vitamin D was considered to usually be the endogenous synthesis in the skin. About 30 min of daily sun exposure of the arms and face without sunscreen could usually provide the daily vitamin D needs (Holick, 1994). Subjects not synthesising vitamin D because of factors such as latitude, season (particularly winter at latitudes higher than 42°), ageing, skin pigmentation, clothing, or sunscreen use, were recommended to consume the RNI. WHO/FAO mentioned the age-related decline in the rate of vitamin D synthesis in the skin, in the rate of vitamin D hydroxylation and in the response of target tissues such as bone (Holick, 1994; Shearer, 1997). WHO/FAO also mentioned studies in older adults, including institutionalised subjects or inpatients with low sun exposure, reporting on 'low' 25(OH)D and elevated PTH or ALP concentrations, decline in bone mass and increase in the incidence of hip fractures (Chapuy and Meunier, 1997; Dawson-Hughes et al., 1997). The recommended nutrient intakes for adults were set at 5 µg/day (19–50 years), 10 µg/day (51–65 years) and 15 µg/day (> 65 years).

The French food safety agency (Afssa, 2001) estimated vitamin D requirements to be 10-15 µg/day from the minimal amounts needed to prevent or correct deficiency (Holick, 1994, 1998; Glerup et al., 2000), and estimated endogenous production to cover 50–70% of these requirements in case of 'normal' sun exposure (i.e. about 5–7 µg/day), thus the reference value was set at 5 µg/day. For adults aged 75 years and over, sun exposure was reported to be frequently insufficient (particularly in women in summer), intestinal absorption to be reduced and endogenous production to be less efficient (Dawson-Hughes, 1996). Considering seasonal changes in 25(OH)D concentrations, and PTH concentrations and bone health in older adults (Dawson-Hughes, 1996; Cynober et al., 2000), the reference value was set at 10–15 µg/day. This was higher than the spontaneous intake observed at that time in France (ESVITAF, 1986; Hercberg et al., 1994), therefore the consumption of supplements under medical supervision or of fortified foods was discussed. The importance of calcium intake was also stressed.

SCF (1993) considered serum 25(OH)D concentration ranges of 25–100 nmol/L (whole population) and 25–50 nmol/L (older and institutionalised people) as advisable. The dietary vitamin D intake needed to attain serum 25(OH)D concentration of 25–100 nmol/L was considered to depend on e.g. latitude, climate, air pollution, social and ethnic groups in Europe, and considered this intake not to be essential for healthy adults with appropriate calcium and phosphate intake and sun

[14] Recommended Dietary Allowance.
[15] Estimated Average Requirement.

exposure (Markestad and Elzouki, 1991). The SCF lacked data on the effect of dietary vitamin D on 25(OH)D concentrations of non-pregnant young adults. Based on studies on older adults (MacLennan and Hamilton, 1977; Toss et al., 1983), an intake of 10 µg/day was considered to maintain 25(OH)D concentrations of 25–100 nmol/L, even in case of minimal endogenous synthesis. For adults aged 18-64 years, the acceptable range of intake was 0–10 µg/day (the highest value being set in case of minimal endogenous vitamin D synthesis). Because of lack of sun exposure and the decline with age of endogenous vitamin D synthesis, the SCF considered older adults (65 years and over) and institutionalised people to require 10 µg/day of vitamin D to maintain 25(OH)D concentrations of 25-50 nmol/L (MacLennan and Hamilton, 1977; Toss et al., 1983).

The UK is currently revising the DRVs for vitamin D (DH, 1991). Based on data on musculoskeletal health outcomes (rickets in infants and children, osteomalacia in adults, risk of falling in adults aged more than 50 years, muscle strength and function in young people and adults), a draft Reference Nutrient Intake (RNI) of 10 µg/day was set for the UK population aged four years and over (SACN, 2015). This was considered as the amount needed throughout the year by 97.5% of the population to maintain 25(OH)D concentrations of at least 25 nmol/L (as set by (DH, 1998)) when UV-B irradiation is minimal. It also applies to minority ethnic groups with darker skin.

An overview of DRVs for vitamin D for adults is presented in Table 1.

Table 1: Overview of Dietary Reference Values for vitamin D for adults

	SACN (2015)	D-A-CH (2015b)	NCM (2014)	NL (2012)	IOM (2011)	WHO/FAO (2004)	Afssa (2001)	SCF (1993)[(h)]	DH (1991)[(i)]
Age (years)	≥ 18	≥ 19	18–74	18–69	19–70	19–50	20–74	18–64	19–64
DRV (µg/day)	10[(a)]	20[(b)]	10[(c)]	10[(b)]	15[(e)]	5[(f)]	5[(g)]	0–10	0
Age (years)						51–65			
DRV (µg/day)						10[(e)]			
Age (years)			≥ 75	≥ 70	≥ 71	≥ 66	≥ 75	≥ 65	≥ 65
DRV (µg/day)			20[(d)]	20[(d)]	20[(e)]	15[(f)]	10–15	10	10

(a): draft PRI
(b): AI in case of lack of endogenous synthesis.
(c): PRI assuming some endogenous vitamin D synthesis. PRI of 20 µg/day in case of little or no sun exposure during the summer season.
(d): PRI.
(e): PRI considering minimal sun exposure.
(f): PRI in case of no endogenous vitamin D synthesis.
(g): Populations with 'normal' sun exposure.
(h): Acceptable range of intake. Zero in case of adequate endogenous synthesis, 10 µg/day for younger adults in case of minimal endogenous synthesis, or for older adults aged 65 years and over.
(i): DRVs currently being revised.
NL: the Netherlands.

4.2. Infants and children

D-A-CH (2015b) considered that infants reach a serum 25(OH)D concentration of at least 50 nmol/L with an intake of 10 µg/day (Wagner et al., 2006; Wagner et al., 2010), which was set as the AI, achieved through supplementation, independent of vitamin D endogenous synthesis and intake through consumption of breast milk or formulas. For older children, a serum 25(OH)D concentration of at least 50 nmol/L was considered to be achieved with an intake of 5–10 µg/day (Viljakainen et al., 2006b). However, a higher value of 20 µg/day was set as the AI for all children after one year given the lack of sun exposure (Cashman et al., 2011a) and vitamin D supplementation was recommended in winter time for children aged up to two years (Wabitsch et al., 2011).

The Nordic Council of Ministers (2014) set a RI of 10 µg/day up to the age of two years, based on rickets prevention (Markestad, 1983; Ala-Houhala, 1985; Specker et al., 1992) and the low sun exposure in Nordic countries. For older children, the vitamin D intake required for serum 25(OH)D concentration above 50 nmol/L in Danish adolescent girls throughout winter was shown to be partly dependent on the status in early autumn (Andersen et al., 2013). A meta-regression analysis on data on children and young adults (Section 5.3.1.) was used to set the RI at 10 µg/day, assuming some vitamin D endogenous synthesis during summer outdoor activities.

The Health Council of the Netherlands (2012) used data on the effect of 7.5–10 µg/day supplemental vitamin D for rickets prevention (Lerch and Meissner, 2007) and assumed a sufficient calcium intake to set an AI of 10 µg/day for children aged up to four years. As most young children do not consume sufficient vitamin D and they should be protected against the sun, the Council advised all young children to take a 10 µg/day vitamin D supplement. Above four years, an AI of 10 µg/day was also set, and fair-skinned children sufficiently exposed to sunlight and with a varied diet (including low-fat margarine, cooking fats and oils) were not considered to require supplemental vitamin D.

IOM (2011) (Appendix B) considered that data were insufficient to establish an EAR for infants and that the low breast milk vitamin D concentration could not be used to set requirements. In infants, an intake of 10 µg/day was associated with no clinical deficiency and a serum 25(OH)D concentration generally above 50 nmol/L (Greer et al., 1982; Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988b; Greer and Marshall, 1989; Hollis and Wagner, 2004b). Thus, 10 µg/day was chosen as the AI, assuming an early supplementation of breast-fed infants and a gradual increase in formula intake in the other infants. For the age 1–18 years, IOM assumed a normal distribution of requirements and minimal sun exposure to set the same EAR and RDA as for adults aged less than 70 years (i.e. 10 and 15 µg/day respectively).

WHO/FAO (2004) considered infants to be at risk for vitamin D deficiency because of their high skeletal growth, particularly breast-fed infants because of the low vitamin D concentration in breast milk (Specker et al., 1985) and low sun exposure. Sporadic cases of rickets in Northern cities, almost always in breast-fed infants (Binet and Kooh, 1996; Brunvand and Nordshus, 1996; Gessner et al., 1997; Pettifor and Daniels, 1997), and the increased need for $1,25(OH)_2D$ at puberty (Aksnes and Aarskog, 1982) were mentioned. Adolescents were considered to usually have sufficient sun exposure to synthesize vitamin D, and vitamin D produced in summer and early autumn to be stored mainly in adipose tissue (Mawer et al., 1972), thus available for winter time. However, 'low' vitamin D stores during adolescence may occur (Gultekin et al., 1987). WHO/FAO set a recommended nutrient intake of 5 µg/day for infants and children with insufficient vitamin D synthesis (e.g. during winter at latitudes higher than 42°).

Afssa (2001) set the reference value at 20–25 µg/day for infants, taking into account the frequency of rickets in some French regions and of 'low' 25(OH)D concentrations at the end of winter. The reference values were set at 10 µg/day (1–3 years), and then at 5 µg/day (4–19 years) based on the same considerations as for adults. Supplementation of breast-fed and formula-fed infants (10-20 µg/day), of children aged 18 months-five years during winter (10–20 µg/day), and of adolescents during winter and with low sun exposure (Zeghoud et al., 1995) was advised.

SCF (1993) considered the incidence of rickets in unsupplemented infants and serum 25(OH)D concentrations in supplemented and unsupplemented infants (Poskitt et al., 1979; Garabedian et al., 1991). The SCF considered that infants 6–11 months should consume at least 10 µg/day and possibly up to 25 µg/day (Garabedian et al., 1991), and that most children aged four years and over, but maybe not those aged 1–3 years, had enough sun exposure for an adequate vitamin D synthesis. Thus, the SCF set a reference value of 10 µg/day for children 1–3 years, then ranges of 0-10 (4-10 years) and 0–15 (11–17 years) µg/day, the higher end of the ranges applying in case of minimal endogenous synthesis.

The UK is currently revising the DRVs for vitamin D (DH, 1991). There were insufficient data to set RNI for infants and children aged 0–3 years (SACN, 2015). Draft 'safe intakes' were set at 8.5-10 µg/day for ages 0 to < 1 year (including exclusively breastfed infants) and 10 µg/day for ages 1 to < 4 years. A draft RNI of 10 µg/day was set for subjects aged four years and over (Section 4.1.).

An overview of DRVS for vitamin D for infants and children is presented in Table 2.

Table 2: Overview of Dietary Reference Values for vitamin D for children

	SACN (2015)[a]	D-A-CH (2015b)[b]	NCM (2014)[c]	NL (2012)[d]	IOM (2011)	WHO/FAO (2004)	Afssa (2001)	SCF (1993)	DH (1991)[j]
Age (months)	0–< 12	0–< 12	6–12	0–< 12	6–12	7–12	6–12	6–11	7–12
DRV (µg/day)	8.5-10	10	10	10	10[e]	5[g]	20–25[h]	10–25	7
Age (years)	1-17	1–18	1–18	1–18	1–18	1–18	1–3	1–3	1–3
DRV (µg/day)	10	20	10	10	15[f]	5[g]	10	10	7
Age (years)							4–19	4–10	4–18
DRV (µg/day)							5	0–10[i]	0
Age (years)								11–17	
DRV (µg/day)								0–15[i]	

(a): draft reference values ('safe intakes" for the age 0–< 4 years, RNI afterwards).
(b): AIs set considering a lack of endogenous vitamin D synthesis. Vitamin D supplementation of infants, and of children aged up to two years during winter, was recommended.
(c): PRI assuming some endogenous vitamin D synthesis.
(d): AIs. Vitamin D supplementation (10 µg/day) of young children was recommended.
(e): AI.
(f): PRI considering minimal sun exposure.
(g): PRI in case of no endogenous vitamin D synthesis.
(h): Based on the summary table of Afssa (2001). Supplementation of infants (10–20 µg/day), of children (18 months-five years) during winter (10–20 µg/day), and of adolescents during winter and with low sun exposure was advisable.
(i): Acceptable ranges of intake. Zero in case of adequate endogenous synthesis, the higher end of the range in case of minimal endogenous synthesis.
(j): DRVs currently being revised. DRVs to be met by supplementation up to at least two years of age.
NL: the Netherlands.

4.3. Pregnancy and lactation

According to D-A-CH (2015b), maternal serum 25(OH)D concentration influences that of the fetus (Hollis and Wagner, 2004a; Wagner et al., 2008a). The vitamin D concentration in breast milk can be influenced by intake (Hollis and Wagner, 2004b, 2004a; Wagner et al., 2006) but with high doses up to 160 µg/day (Wagner et al., 2006; Hollis et al., 2011), which were not considered advisable by D-A-CH (Wagner et al., 2008b). The same AI as that for non-pregnant non-lactating women was thus set, i.e. 20 µg/day in case of lack of endogenous vitamin D synthesis.

The Nordic Council of Ministers (2014) considered the marked increase in serum $1,25(OH)_2D$ concentration during pregnancy, a correlation between maternal and neonatal vitamin D status (Markestad, 1983), and lower winter serum 25(OH)D concentrations in pregnant Nordic women (Bjorn Jensen et al., 2013; Brembeck et al., 2013). The Council also considered the 'normal' serum 25(OH)D concentrations in pregnant women supplemented with 10 µg/day vitamin D (Markestad et al., 1986), the improved vitamin D status at term by supplementation during pregnancy (Cranney et al., 2007; De-Regil et al., 2012; Lamberg-Allardt et al., 2013), and the limited data on health outcomes. Thus, the previous RI for pregnant or lactating women, i.e. 10 µg/day, was maintained.

The Health Council of the Netherlands (2012) advised vitamin D supplementation particularly for pregnant women with light skin and insufficient sun exposure, or those with dark skin (10 µg/day,

maybe even prior to pregnancy) and noted the low vitamin D concentration in breast milk (IOM, 2011). The Council applied the same AI for pregnant or lactating women as for other young women

IOM (2011) (Sections 5.1.2. and 5.1.3., Appendix B) found (i) insufficient evidence on the association between maternal serum 25(OH)D concentration and BMD during pregnancy, (ii) no effect of maternal 25(OH)D concentration in pregnancy on fetal calcium homeostasis or skeletal outcomes, (iii) negative skeletal outcomes in the newborn below the EAR-type value (40 nmol/L, Section 4.1.) for maternal 25(OH)D concentration and (iv) no reduced skeletal BMC in children above the RDA-type value (50 nmol/L, Section 4.1.) for maternal 25(OH)D concentration (Delvin et al., 1986; Javaid et al., 2006; Cranney et al., 2007; Viljakainen et al., 2010). The IOM also considered that neither maternal BMD nor maternal or fetal serum 25(OH)D concentrations could be used to set reference values for vitamin D during lactation. IOM (2011) noted that there is no evidence that the vitamin D requirement of lactating adolescents or women differs from that of non-lactating females in relation to maternal or child outcomes. Thus, the same EAR and RDA were set for pregnant or lactating women as for non-pregnant non-lactating women.

WHO/FAO (2004) considered the limited impact of changes in vitamin D metabolism during pregnancy on maternal requirements, the vitamin D transfer from mother to fetus, and the use of conventional prenatal vitamin D supplements to ensure adequate vitamin D status. WHO/FAO estimated that there was no direct role for vitamin D in lactation because of the regulation of increased calcium needs by the PTH-related peptide (Sowers et al., 1996; Prentice, 1998) and the lack of evidence of any change in vitamin D metabolites during lactation (Kovacs and Kronenberg, 1997; Sowers et al., 1998). Vitamin D concentration in breast milk was considered as low (Specker et al., 1985), and the rare cases of nutritional rickets were almost always observed in breast-fed infants not exposed to the sun (Binet and Kooh, 1996; Brunvand and Nordshus, 1996; Gessner et al., 1997; Pettifor and Daniels, 1997). Evidence was lacking for an increased calcium or vitamin D transfer in milk after supplementation in lactating mothers (Sowers et al., 1998). Therefore, the same recommended nutrient intake of 5 µg/day was applied for pregnant and lactating women and for other younger women (19–50 years).

Afssa (2001) considered that pregnant women in France may have a deficient vitamin D status at the end of pregnancy, particularly in winter or early spring, even in the South of France. Vitamin D supplementation (25 µg/day during the last trimester, or a single dose of 5 mg at the seventh month) was also mentioned. The reference value of pregnant or lactating women was set at 10 µg/day.

The SCF (1993) considered that usual sun exposure in Europe may be insufficient to cover vitamin D needs, especially during the last trimester of pregnancy and at the end of winter, and that the ensuing vitamin D deficiency would affect mother and newborn (as neonatal vitamin D stores depend on maternal ones). The SCF (1993) set a PRI of 10 µg/day to maintain 25(OH)D concentrations of pregnant and lactating women (Cockburn et al., 1980; Greer et al., 1981).

The UK is currently revising the DRVs for vitamin D (DH, 1991). The draft RNI of 10 µg/day proposed for subjects aged four years and over (Section 4.1.) also applies to pregnant and lactating women (SACN, 2015).

An overview of DRVs for vitamin D for pregnant and lactating women is presented in Table 3.

Table 3: Overview of Dietary Reference Values for vitamin D for pregnant and lactating women

	SACN (2015)[a]	D-A-CH (2015b)[b]	NCM (2014)[c]	IOM (2011)[c]	NL (2012)[d]	WHO/FAO (2004)[c]	Afssa (2001)	SCF (1993)[c]	DH (1991)[e]
Pregnancy (µg/day)	10	20	10	15	10	5	10	10	10
Lactation (µg/day)	10	20	10	15	10	5	10	10	10

(a): draft RNI.
(b): AI in case of lack of endogenous synthesis of vitamin D.
(c): PRI.
(d): AI.
(e): Reference values currently being revised. Reference values to be met by supplementation.
NL: the Netherlands.

5. Criteria (endpoints) on which to base Dietary Reference Values

The Panel considered serum 25(OH)D concentration as a useful biomarker of vitamin D intake (in a population with low exposure to UV-B irradiation) and of vitamin D status in children and adults (Section 2.4.6.). The Panel also considered that serum 25(OH)D concentration represents total vitamin D from exposure to both UV-irradiation (cutaneous synthesis) and dietary sources (Section 2.3.3.). The Panel considered that the association between vitamin D intake and status for the purpose of deriving DRVs for vitamin D should be assessed under conditions of minimal endogenous vitamin D synthesis (Section 2.3.1.). As indicated previously (Sections 2.4.1. and 4), there is an ongoing debate about the optimal range of serum 25(OH)D concentration and the cut-off values for defining deficiency, insufficiency and sufficiency.

Thus, the Panel reviewed data first on serum 25(OH)D *concentration* and *health* outcomes (Section 5.1.)), irrespective of the analytical method applied to measure serum 25(OH)D concentration (Section 2.4.1.). Then, the Panel reviewed data on vitamin D *intake* (from supplements) and *health* outcomes (Section 5.2.). Finally, the Panel reviewed and assessed data on the relationship between vitamin D *intake* (from food and supplements) and serum 25(OH)D *concentration* under conditions of minimal endogenous synthesis, and on factors potentially influencing this relationship (Section 5.3., Appendices C and D).

5.1. Serum 25(OH)D concentration and health outcomes

5.1.1. Serum concentration

The active metabolite $1,25(OH)_2D$ in association with VDR has a biological function not limited to bone, intestine, kidneys and parathyroid glands, but throughout the body, regulating many functions (Section 2.3.6.). The Panel thus considered the relationships between vitamin D status, assessed by *serum 25(OH)D concentration*, and various health outcomes (musculoskeletal or non musculoskeletal), to evaluate whether they might inform the setting of DRVs for vitamin D. This assessment was undertaken irrespectively of the analytical method applied to measure serum 25(OH)D concentration (Section 2.4.1.).

The review of data on serum 25(OH)D concentration and *musculoskeletal* health outcomes in adults and children is first described (Section 5.1.1.). Then, the Panel reviewed data on serum 25(OH)D concentration and health outcomes in *pregnancy* (Section 5.1.2.) and *lactation* (Section 5.1.3.). Finally, an overview of available data on serum 25(OH)D and *non-musculoskeletal* health outcomes is given (Section 5.1.4.).

- For *all of these outcomes*, the Panel took a starting point in the results and conclusions from the report by IOM (2011) (Section 4, Appendix B). This report by the IOM was based (i) on

the systematic review (of RCTs (mainly), prospective cohort, case-control and before-after studies published in 1966–2006) by Cranney et al. (2007) on effectiveness and safety of vitamin D in relation to bone health, (ii) on another systematic review (of RCTS, non-randomised comparative studies, cohort and nested case-control studies and systematic reviews) by Chung et al. (2009) on vitamin D and/or calcium and various health outcomes, which focused however on RCTs published in 2006–2008 in relation to bone health outcomes to update the review by Cranney et al. (2007), and (iii) on additional literature search.

- For *all of these outcomes*, the Panel also considered the report of the Agency for Healthcare Research and Quality (AHRQ) by Newberry et al. (2014), which is an update of Chung et al. (2009) for the period 2008–2013 with regard to data on vitamin D intake (and status) with or without calcium. The Panel considered as well the draft report by SACN (2015) as submitted for public consultation and that served as a basis for updating the references values for vitamin D in the UK. The draft report by SACN (2015)took the report by IOM (2011) as a starting point and reviewed human studies published up to 2014. For *musculoskeletal health outcomes*, the Panel also considered the systematic literature review (of systematic reviews (mainly) and RCTs published in 2000-2012) by Lamberg-Allardt et al. (2013) on vitamin D intake and status and health (including safety), which tried to identify a serum 25(OH)D concentration that would reflect sufficient vitamin D status and served as a basis for updating the reference values for vitamin D for the Nordic Nutrition Recommendations 2012 (Nordic Council of Ministers, 2014) (Section 4).

- For its literature search related to *musculoskeletal* health outcomes in adults and children, as well as health outcomes in *pregnancy* and *lactation*, the Panel considered pertinent *primary studies* published from 2010 (after the IOM report) onwards until March 2015 in PubMed and/or as identified in Newberry et al. (2014) and/or SACN (2015), on the possible relationship between vitamin D status and health outcomes, with the aim to identify a serum 25(OH)D concentration to be used for deriving the DRVs for vitamin D. (Also, using the same approach, the Panel considered pertinent primary studies on vitamin D intake and health outcomes, see Section 5.2.).

Regarding the design of the primary studies considered, the Panel focused on intervention studies and prospective observational studies in healthy subjects, i.e. excluding cross-sectional studies (except for osteomalacia), case reports and ecological studies. The Panel notes that, in observational studies, positive, inverse, or lack of associations between 25(OH)D concentrations and musculoskeletal health outcomes might be biased because of uncertainties in the methodology for measuring serum 25(OH)D concentrations or confounded by factors that have not been properly addressed. In the following sections, for each *musculoskeletal* health outcome in adults and children, as well as each health outcomes in *pregnancy* and *lactation*, first the *intervention studies* and then the *prospective observational studies* are described individually, and finally, an *overall discussion and conclusion* by health outcome is provided.

With the aim of setting DRVs for vitamin D, the Panel considered studies on vitamin D intake from food and/or daily or weekly supplementation using doses up to the UL for the respective population group (e.g. for adults: 100 µg/day) (EFSA NDA Panel, 2012a), and excluded studies reporting on lower frequency of consumption (e.g. monthly, once per trimester, or yearly administration).

5.1.2. Serum 25(OH)D concentration and musculoskeletal health outcomes

The Panel considered musculoskeletal health outcomes to include BMD/BMC, risk of osteomalacia or of rickets (Section 2.2.2.1.), fracture risk, risk of falls/falling, muscle strength/muscle

function/physical performance, and calcium absorption. Markers of bone turnover (i.e. of bone formation and resorption) were not considered (Section 2.4.5.).

In the context of reviewing the available evidence on vitamin D status and musculoskeletal health outcomes with the aim of identifying a serum 25(OH)D concentration that may indicate adequate musculoskeletal health and thus may be used for the setting of DRVs for vitamin D, the Panel decided to consider available data on bone measurements (BMC, BMD) in children and adults obtained via different techniques (e.g. dual-energy X-ray absorptiometry DXA or peripheral quantitative computed tomography pQCT, Appendix A) and after an appropriate study duration (e.g. at least one year (EFSA NDA Panel, 2012b)).

5.1.2.1. Adults

5.1.2.1.1. Bone mineral density/bone mineral content (BMD/BMC)

IOM (2011) (Section 4 and Appendix B) underlined that results from RCTs did not show an association between serum 25(OH)D concentration and BMD or bone loss. The IOM considered, however, that the majority of observational studies in postmenopausal women and older men supported an association between serum 25(OH)D concentration and BMD or change in BMD, particularly at the hip sites, and that 25(OH)D concentrations that were associated with an increase of bone loss at the hip ranged from < 30 to 80 nmol/L.

Lamberg-Allardt et al. (2013) based their conclusions about the possible relationship between 25(OH)D concentration and BMD or BMC in older adults on Cranney et al. (2007) and Chung et al. (2009) and their conclusions were in agreement with those derived by IOM (2011). Newberry et al. (2014) did not specifically report on the relationship between 25(OH)D concentration and BMC/BMD in adults beyond the conclusions of IOM (2011). With regard to bone health indices in adults aged 50 years and over, SACN (2015) additionally considered a systematic review by Reid et al. (2014) that included 23 studies (most of which were published between 1991 and 2009; four of the seven more recent studies were on patients or institutionalised subjects), two intervention studies (Kärkkäinen et al., 2010; Macdonald et al., 2013) and one prospective cohort study (Ensrud et al., 2009). However, no overall conclusion was drawn on the association between serum 25(OH)D concentration and risk for increase of bone loss.

The Panel retrieved 14 intervention and prospective observational studies in non-institutionalised adults, reporting on BMD/BMC in relation to 25(OH)D concentrations and that were published after the report by IOM (2011). In the following section, the *six intervention studies* and then the *eight prospective observational studies* are described individually. The results are then summarized, and an *overall conclusion on BMD/BMC* in adults is provided.

RCTs with vitamin D supplementation

In a double-blind one-year RCT performed in Norway by Jorde et al. (2010), overweight men and women (21–70 years) received 500 µg vitamin D_3 per week (equivalent to 71 µg/day) (DP group n = 132), or placebo (PP group, n = 142). All subjects were given 500 mg/day calcium and 202 subjects completed the study. Mean (standard deviation SD) serum 25(OH)D concentrations increased from 58 (20) to 100 (20) nmol/L in the DP group and remained unchanged in the PP group (58 (20) nmol/L). After one year, there were no significant differences between the two groups regarding change in BMD (lumbar spine and hip). **The Panel notes** that raising mean 25(OH)D concentration from 58 to 100 nmol/L by weekly high dose supplementation with vitamin D for one year did not have an effect on BMD in these healthy overweight and mostly vitamin D sufficient subjects with an adequate calcium supply and who covered a large age range.

In a one-year RCT by Islam et al. (2010), 200 apparently healthy young female factory workers (16-36 years) in Bangladesh received either: (1) daily 10 µg vitamin D[16]; (2) daily 10 µg vitamin D + 600 mg calcium; (3) 10 µg vitamin D and other micronutrients + 600 mg calcium; or (4) placebo. These women worked from dawn to dusk on all days of the week and wore concealing clothing (hands and faces uncovered). Mean 25(OH)D concentration was between 35 and 38 nmol/L among the groups at baseline, but was significantly ($p < 0.001$) higher in the three supplemented groups than in the placebo group (69 vs 36 nmol/L) at the end of the study. After adjustments for potential confounders, BMD and BMC increased significantly at the femoral neck ($p < 0.001$) and at the greater trochanter and Ward's triangle ($p < 0.05$) in the supplemented groups compared with placebo, but there was no significant difference between groups at the lumbar spine (L2–L4). **The Panel notes** that raising mean 25(OH)D concentration from 35–38 nmol/L up to 69 nmol/L in these young Bangladeshi women with low sun exposure by vitamin D supplementation (with or without calcium) for one year was associated with a significant increase in BMD at the femoral neck, greater trochanter and Ward's triangle, but not at the lumbar spine.

In a randomly selected subsample of 593 subjects from a randomised population-based open trial with a three-year follow-up in 3,432 women (aged 66–71 years) in Finland (Kärkkäinen et al., 2010), the intervention group (n = 287) received daily 20 µg vitamin D_3 + 1,000 mg calcium for three years, while the control group (n = 306) received neither supplementation nor placebo. The respective mean calcium intakes were 988 and 965 mg/day at baseline. The respective mean (SD) 25(OH)D concentrations were 50.1 (18.8) and 49.2 (17.7) nmol/L at baseline. At the end of the trial, serum 25(OH)D was significantly higher in the intervention group as compared to the control group (74.6 (21.9) vs 55.9 (21.8) nmol/L, $p < 0.001$). In the intention-to-treat (ITT) analysis, total body BMD (n = 362) increased significantly more in the intervention group than in the control group (0.84% vs 0.19%, $p = 0.011$) and the BMD decrease at Ward's triangle was lower in the intervention group (- 2.69% vs - 2.83%, $p = 0.003$). BMD changes at the lumbar spine, femoral neck, trochanter, and total proximal femur were not statistically different between groups. The women who were adherent (i.e., those who took at least 80% of their supplementation) 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%, $p < 0.0001$) than in the control group. Further, total body BMD increased more in the intervention group (1.31% vs 0.19%, $p = 0.002$). In contrast, the increase in lumbar spine BMD was lower in the intervention group than in the control group (0.67% vs 0.76%, $p = 0.033$). **The Panel notes** that raising mean 25(OH)D concentration from 50 nmol/L to 75 nmol/L by daily vitamin D and calcium supplementation for three years was associated with a significantly higher increase in total BMD in these women and, in subjects that adhered to the protocol, with a significantly lower bone loss in femoral neck, Ward's triangle, trochanter and total proximal femur, but a significantly lower increase in lumbar spine BMD compared to the control group. The Panel also notes that all analyses were unadjusted.

In an 18-months RCT with a factorial design in Australia by Kukuljan et al. (2011), 180 Caucasian men aged 50–79 years were randomised to: fortified milk (400 mL/day of milk containing 1,000 mg/day calcium and 20 µg/day vitamin D_3); exercise + fortified milk; exercise; or control (no milk, no exercise). Mean baseline serum 25(OH)D concentrations averaged 86.3 ± 36 nmol/L across the groups, in which no, one and 17 participants had serum 25(OH)D concentrations below 12.5 nmol/L, of 12.5–25 nmol/L and of 25–50 nmol/L, respectively. Serum 25(OH)D concentrations increased by an average of 21 nmol/L in the fortified milk compared with the two non-fortified milk groups after 12 months ($p < 0.001$), with no further increases observed at 18 months. Changes in BMD, bone structure, and strength at the lumbar spine, proximal femur (femoral neck), mid-femur, and mid-tibia were measured. There were no exercise-by-fortified milk interactions at any skeletal site. Main effect analysis showed that exercise led to a net gain in femoral neck section modulus (a measure for bending strength) and lumbar spine trabecular BMD, but there were no main effects of

[16] Personal communication from one author: vitamin D_3.

the fortified milk at any skeletal site. **The Panel notes** that raising mean 25(OH)D concentration from about 86 to 107 nmol/L by providing vitamin D_3 (with calcium) to these mostly replete men for 18 months did not enhance BMD. This suggests that other factors may confound the relationship between vitamin D intake, serum 25(OH)D and BMD or that, above a certain 25(OH)D concentration, there is no effect of additional calcium and vitamin D on BMD.

In a two-year double-blind RCT in the US, Nieves et al. (2012) investigated the effect of 25 µg/day vitamin D_3 supplementation vs placebo on bone loss in postmenopausal African American women (mean age about 62 years) (ITT: n = 103) and the influence of polymorphisms in the gene encoding VDR (Section 2.2.1., 2.3.6. and 2.5.). All women received calcium supplementation (total intake 1,000 mg/day). Mean (± SD) baseline 25(OH)D concentrations were 29 ± 13 and 29 ± 14 nmol/L in the intervention (n = 55) and placebo (n = 48) groups, respectively, and in 50% of the subjects, 25(OH)D concentration was below 25 nmol/L. After two years, serum 25(OH)D significantly increased by 27.5 nmol/L in the intervention group ($p < 0.001$), but did not change in the placebo group. Two-year changes in spine or hip BMD did not significantly differ between groups at any skeletal site. When the entire population was divided according to *Fok*1 polymorphism (that has been associated with BMD in postmenopausal women), there were no significant differences in the 25(OH)D response to vitamin D supplementation by genotype. Despite similar elevations in 25(OH)D, femoral neck BMD was only responsive to vitamin D supplementation in *FF* subjects (n = 47), not in *Ff/ff* subjects (n = 31). **The Panel notes** that, in these postmenopausal African American women, raising mean 25(OH)D concentration from about 29 to 56 nmol/L by vitamin D supplementation was not associated with significantly different two-year changes in spine or hip BMD compared with the placebo group, both groups having a mean baseline 25(OH)D concentration of 29 nmol/L and sufficient calcium supply. The Panel also notes that the possible relationship between baseline or follow-up 25(OH)D concentration and BMD may depend among other factors on genetic predisposition. In this context, the Panel notes that, with regard to the *Fok*1 polymorphism, the reported frequency of the *FF* genotype among various populations was reported to be between 40 and 50% (Laaksonen et al., 2004; Sanwalka et al., 2013).

In a one-year double-blind RCT in Scotland, Macdonald et al. (2013) determined whether daily vitamin D_3 supplementation compared with placebo affects BMD change in healthy Caucasian postmenopausal women aged 60–70 years (ITT: n = 264). Mean intakes of calcium and vitamin D from food and other supplements amounted to around 1.3 g/day and 5 µg/day at baseline in all groups. Total mean vitamin D intake (i.e. with food and all supplements) amounted to about 5, 15, and 30 µg/day in the placebo (n = 90), 10 µg supplemented (n = 84) and 25 µg supplemented (n = 90) groups, respectively. Mean (± SD) baseline 25(OH)D was 33.8 ± 14.6 nmol/L. The 25(OH)D changes were - 4.1 ± 11.5 nmol/L, + 31.6 ± 19.8 nmol/L, and + 42.6 ± 18.9 nmol/L in the placebo, 10 µg, and 25 µg groups, respectively. After adjustments for potential confounders, mean BMD loss at the hip, but not lumbar spine, was significantly less for the 25 µg vitamin D group (0.05% ± 1.46%) compared with the 10 µg vitamin D or placebo groups (0.57% ± 1.33% and 0.60% ± 1.67%, respectively) ($p < 0.05$). Neither at baseline nor at the final visit, significant associations between serum 25(OH)D and mean BMD were found for either total hip or lumbar spine. **The Panel notes** that raising mean 25(OH)D concentration from about 34 to 65 or 76 nmol/L by two supplemental doses of vitamin D for one year in these postmenopausal women did not result in corresponding effects (i.e. in a dose-response relationship) on BMD when calcium supply is sufficient. This suggests that other factors may confound the relationship between vitamin D intake, serum 25(OH)D and BMD, and that 25(OH)D concentrations above 34 nmol/L are not associated with BMD.

Prospective observational studies

In a five year calcium supplementation study in Australia, Bolland et al. (2010) (Sections 5.1.1.1.1.3. and 5.1.1.1.4.1.) examined the association between baseline serum 25(OH)D concentration and multiple health outcomes in 1,471 community dwelling women (mean age

74 years). Fifty percent of women had a seasonally adjusted 25(OH)D concentration < 50 nmol/L and these women were significantly older, heavier, and less physically active and had more comorbidities than women with a seasonally adjusted 25(OH)D concentration ≥ 50 nmol/L. After adjustments for potential confounders (including treatment allocation to calcium or placebo), women with a seasonally adjusted baseline 25(OH)D concentration < 50 nmol/L and those with 25(OH)D concentrations ≥ 50 nmol/L did not show any difference in change in bone density (lumbar spine, total femur, total body). **The Panel notes** that this study of community-dwelling older women showed no difference in BMD change in those with a seasonally adjusted 25(OH)D concentration < 50 nmol/L compared with those with 25(OH)D concentrations ≥ 50 nmol/L over a five year period.

In a cohort of 1,097 healthy peri- or postmenopausal Caucasian Danish women (45–57 years, median: 51 years) with a 16-year follow-up, Rejnmark et al. (2011) investigated the association of tertiles of PTH concentrations (upper tertile ≥ 4.5 pmol/L) with BMD (assessed at the 10-year follow-up) stratified according to baseline 25(OH)D concentrations < 50 nmol/L, at 50–80 nmol/L, or > 80 nmol/L, after adjustments for potential confounders. Mean baseline plasma 25(OH)D was 65 ± 31 nmol/L. Within the group of women with plasma 25(OH)D < 50 nmol/L at baseline, high (≥ 4.5 pmol/L), compared to low (< 4.5 pmol/L), PTH concentrations were associated with a significantly larger decrease in lumbar spine BMD between baseline and the 10-year visit (- 5.6 ± 7.0% vs - 3.4 ± 7.0%, p = 0.01) after adjustments for potential confounders. In contrast, high vs low PTH concentrations were not associated with bone loss rates at the lumbar spine in women with 25(OH)D concentrations of 50–80 nmol/L or in women with 25(OH)D concentrations > 80 nmol/L. However, there was no influence of plasma 25(OH)D concentration on the relationships of PTH with 10-year changes in BMD at the total hip, femoral neck, and whole body. **The Panel notes** that this study indicates that, in these women, a greater 10-year BMD loss at the lumbar spine was associated with a baseline plasma 25(OH)D concentration < 50 nmol/L at higher PTH concentrations and that the relationship between 25(OH)D concentration and BMD depends on PTH.

In a cohort of mobile community-dwelling Chinese men aged 65 years and over (n = 712) with a four-year follow-up, Chan et al. (2011) examined serum 25(OH)D in relation to BMD. Mean baseline 25(OH)D concentration was 78.2 ± 20.5 nmol/L, and respectively 5.9%, 41.5%, and 52.6% had concentration below 50 nmol/L, of 50 to < 75 nmol/L, or 75 nmol/L or higher. After adjustments for potential confounders, there was no association between serum 25(OH)D concentration and four-year percentage change in BMD at total hip, spine, and femoral neck. The results remained unchanged when subjects were divided into quartiles of serum 25(OH)D, i.e. concentration of the first quartile ≤ 63 nmol/L vs concentration > 63 nmol/L. **The Panel notes** that, in this study in men with a mean serum 25(OH)D concentration of about 78 nmol/L at baseline, no association was found between baseline serum 25(OH)D concentration (continuous variable or over quartiles of < 63 nmol/L up to > 91 nmol/L) and a lower four-year bone loss at any site.

In a cohort study among 2,614 community-dwelling white and black women and men aged ≥ 70 years in the U.S.A., secondary analyses were conducted by Barbour et al. (2012) to determine the average annual change in hip areal BMD (aBMD) by quartiles of 25(OH)D concentration (< 44.5 nmol/L, 44.5–61 nmol/L, 61–79.8 nmol/L, > 79.8 nmol/L; mean baseline value not reported). Blood samples were drawn at year 2, which formed the baseline for this analysis, and hip aBMD was measured at baseline, years 3, 5 or 6, 8, and 10. After adjustments for potential confounders, lower 25(OH)D was associated with greater aBMD loss (p trend = 0.024). Participants in the top 25(OH)D quartile had significantly lower annualised hip aBMD loss (- 0.55%, 95% CI - 0.48 to - 0.62%) compared with those in the lowest quartile (- 0.65%, 95% CI - 0.58 to - 0.72%). **The Panel notes** that, in this study, a baseline serum 25(OH)D concentration below 44.5 nmol/L (lowest quartile) was associated with a 0.1% higher annual hip aBMD loss compared to serum 25(OH)D > 79.8 nmol/L.

In a case-cohort study with a 4.6 year follow-up in the US, Barrett-Connor et al. (2012) tested the hypothesis that combinations of 'low' serum 25(OH)D concentration (< 50 nmol/L), 'low' sex hormones (SH) (bioavailable testosterone (BioT) < 163 ng/dL; bioavailable estradiol (BioE) < 11 pg/mL), and 'high' sex hormone binding globulin (SHBG) (> 59 nmol/L) would have a synergistic effect on total hip BMD loss. Participants were a random subsample of 1,468 men (mean age: 74 years) from a larger prospective cohort study plus 278 men from this cohort with incident non-spine fractures. One quarter of the men had 25(OH)D < 50 nmol/L (mean 38.8 nmol/L). After adjustments for potential confounders, 'low' 25(OH)D in isolation, and 'low' BioT with or without 'low' 25(OH)D, were not significantly related to BMD loss. However, the combination of 25(OH)D < 50 nmol/L with 'low' BioE and/or 'high' SHBG was associated with significantly lower baseline total hip BMD ($p = 0.03$, $p = 0.002$) and higher annualised rates of hip bone loss ($p = 0.007$, $p = 0.0006$), than SH abnormalities alone or no abnormality. **The Panel notes** that the adverse effect of 'low' BioE and/or 'high' SHBG serum concentrations on total hip BMD was more pronounced in older men with baseline serum 25(OH)D concentrations < 50 nmol/L (lowest quartile, mean 38.8 nmol/L), whereas 25(OH)D concentration < 50 nmol/L in isolation was not associated with BMD.

In a population-based cohort of 192 apparently healthy ambulatory older Lebanese men (n = 64) and women (n = 128) aged 65–85 years, with a median four-year follow-up, Arabi et al. (2012) analysed the association of 25(OH)D, PTH and body composition with change in BMD at the lumbar spine, hip (femoral neck, trochanter, total hip), and forearm and subtotal body BMC. For 25(OH)D and PTH, average of baseline and follow-up concentrations were used in the analyses. Mean 25(OH)D concentration was 36.8 ± 16 nmol/L and BMD significantly decreased at all skeletal sites except at the spine. Multivariate analyses of percent changes in BMD (at all skeletal sites) or subtotal body BMC showed that 25(OH)D was not a significant predictor, contrary to changes in body composition and PTH. **The Panel notes** that this study showed no association between serum 25(OH)D and four-year bone loss at the lumbar spine, hip or forearm in a population with a mean serum 25(OH)D concentration of about 37 nmol/L (average of baseline and follow-up).

In a cohort study in Japan, Kitamura et al. (2013) explored the association between serum 25(OH)D concentrations, PTH concentrations and five-year changes in BMD of the lumbar spine and femoral neck in 482 independently living postmenopausal women (mean age, range: 63.1 years, 55-74 years). Their mean baseline serum 25(OH)D concentration was 56 nmol/L. In the serum 25(OH)D quartiles (< 46.5, 46.5 to < 56.1, 56.1 to < 65.1, ≥ 65.1 nmol/L), mean concentrations were 37.5 ± 7.5, 51.2 ± 2.8, 60.3 ± 2.4, and 74.7 ± 7.7 nmol/L, respectively. Mean calcium intake was not significantly different between serum 25(OH)D quartiles (519–536 mg/day). After adjustment for potential confounders, there was no significant association between baseline serum 25(OH)D concentrations (as quartiles) and change in BMD (at either site). **The Panel notes** that this study indicates that, even at a rather low calcium intake, the lowest baseline quartile serum 25(OH)D concentration (< 46.5 nmol/L, mean of about 38 nmol/L) was not associated with a higher five-year postmenopausal bone loss at the lumbar spine or femoral neck.

In a cohort of 922 women during the menopausal transition (mean age 48.5 ± 2.7 years) at five US clinical centers and with an average follow-up of 9.5 years, Cauley et al. (2015) determined if higher 25(OH)D baseline concentration is associated with slower loss of BMD. BMD was measured at each annual visit. The mean 25(OH)D concentration was 54.5 nmol/L; 43% of the women had 25(OH)D concentrations < 50 nmol/L. Changes in lumbar spine and femoral neck BMD across menopause were not significantly associated with serum 25(OH)D concentration. **The Panel notes** that, in this study, baseline serum 25(OH)D concentrations (mean 54.5 nmol/L) were not associated with changes in lumbar spine and femoral neck BMD across menopause.

Conclusions on BMD/BMC in adults

Among the 14 studies identified, most of which were in older non-institutionalised adults, the Panel notes the heterogeneity of study designs, populations and skeletal sites investigated. The Panel considers that the sensitivity of serum concentrations of 25(OH)D in predicting losses in BMD/BMC may be limited because of confounding by a variety of factors (e.g. PTH, genetic factors, sex steroids, body composition, age, sex, calcium intake, life-style factors, baseline values, season of assessment, and possible other yet unknown factors) that have only been partly considered in these analyses. Furthermore, observational studies mostly used single measurements of 25(OH)D concentrations, thus possible long-term changes in 25(OH)D concentration were not considered in the analyses of the relationship with BMD/BMC changes.

Of the six **RCTs** with vitamin D supplementation durations between one and three years**,** two RCTs in women indicated that daily vitamin D and calcium supplementation that led to an increase in mean 25(OH)D concentrations from 35–38 nmol/L to 69 nmol/L (Islam et al., 2010) and from 50 nmol/L to 75 nmol/L (Kärkkäinen et al., 2010), respectively, was associated with a significantly higher increase in BMD compared to the control group. In subjects that adhered to the protocol, raising mean 25(OH)D concentration from 50 nmol/L to 75 nmol/L was also associated with a significantly lower bone loss in femoral neck, Ward's triangle, trochanter and total proximal femur (Kärkkäinen et al., 2010). However, in four RCTs, an increase in serum 25(OH)D concentration from a mean of 29 nmol/L (Nieves et al., 2012), 34 nmol/L (Macdonald et al., 2013), 58 nmol/L (Jorde et al., 2010) and 86 nmol/L (Kukuljan et al., 2011) up to 56 nmol/L, 76 nmol/L, 100 nmol/L and 107 nmol/L, respectively, after vitamin D supplementation or consumption of vitamin D-fortified food (with or without calcium), did not result in a change in BMD.

Of the eight **prospective observational studies**, one reported a 0.1% higher annual hip aBMD loss associated with baseline 25(OH)D concentrations < 45 nmol/L (lowest quartile), as compared to 25(OH)D concentrations above 80 nmol/L (highest quartile) (Barbour et al., 2012). One study found a significant relationship between PTH concentration and 10-year BMD loss at the lumbar spine at baseline serum 25(OH)D concentrations of < 50 nmol/L (Rejnmark et al., 2011). A third study observed an association between annual hip BMD loss and baseline 25(OH)D concentrations < 50 nmol/L (lowest quartile, mean 39 nmol/L) only in subjects with 'low' sex steroid concentrations (Barrett-Connor et al., 2012). However, three studies found no difference in (four or five-year) BMD changes at any sites between baseline serum 25(OH)D concentrations in the lowest quartile (< 46.5 nmol/L, (Kitamura et al., 2013); < 50 nmol/L (Bolland et al., 2010); < 63 nmol/L, (Chan et al., 2011)) and higher concentrations. Two other studies also did not find an association between BMD or BMC losses and serum concentrations of 25(OH)D in populations with mean 25(OH)D of 37 nmol/L (average of baseline and four-year-follow-up) (Arabi et al., 2012) or 55 nmol/L (baseline) (Cauley et al., 2015).

The Panel notes that **two RCTs** (Islam et al., 2010; Kärkkäinen et al., 2010) indicate that BMD may increase when mean serum 25(OH)D concentration increases from about 35–38 to 69 nmol/L in young women and from 50 to 75 nmol/L in older women and that BMD losses at sub-sites may be less pronounced when mean serum 25(OH)D concentration is increased from about 50 to 75 nmol/L in these older women. The Panel also notes that **three observational studies** (Rejnmark et al., 2011; Barbour et al., 2012; Barrett-Connor et al., 2012) suggest that baseline serum 25(OH)D concentrations below 45–50 nmol/L (alone (Barbour et al., 2012) or in combination with high PTH concentration or low' BioE and/or 'high' SHBG (Rejnmark et al., 2011; Barrett-Connor et al., 2012)) may be associated with increased BMD losses at various sites. However, the Panel considers that the majority of both RCTs and observational studies do not report increased BMD/BMC losses at or below similar serum 25(OH)D concentrations (baseline mean or lowest quartile). The Panel notes that other factors can interfere with the association between 25(OH)D and BMD/BMC and thus may contribute to these inconsistencies. The Panel concludes that, altogether, these 13 studies in apparently healthy adults, published after the report by IOM (2011), do not provide sufficient

evidence for a conclusion on a serum 25(OH)D concentration below which there is an increased risk of BMD/BMC loss.

The IOM had considered that results from RCTs did not show an association between serum 25(OH)D concentration and BMD or bone loss, but that the majority of observational studies in postmenopausal women and older men supported an association between serum 25(OH)D concentration and BMD or change in BMD, particularly at the hip sites. IOM also considered that serum 25(OH)D concentrations that were associated with an increase in bone loss at the hip ranged from below 30 to 80 nmol/L. **Taking into account the conclusions of IOM (2011) and the studies published thereafter, the Panel considers** that there is some evidence that the risk of increased BMD/BMC loss in non-institutionalised adults is higher with a serum 25(OH)D concentration below 50 nmol/L.

5.1.2.1.2. Osteomalacia

Only one study (Priemel et al., 2010), considered by **IOM (2011)**, in 675 subjects aged 20-100 years (mean age = 58.7 years in males (n = 401) and 68.3 years in females (n = 274)), provides information on serum 25(OH)D concentrations and osteomalacia (Section 2.2.2.1.) assessed by *post mortem* bone biopsies. These subjects had been residing in Germany and died for reasons not related to cancer, metabolic disorders, or bone diseases. Priemel et al. (2010) assessed bone undermineralisation by pathological accumulation of osteoid, and defined osteomalacia as a ratio of osteoid volume (OV, i.e. bone matrix that is not mineralised) to total bone volume (BV) greater or equal to 2%. Only a few subjects had osteomalacia (OV/BV $\geq$ 2%) at serum 25(OH)D concentrations above 50 nmol/L and no subject had osteomalacia at serum concentrations of at least 75 nmol/L. By further inspecting the graphical presentation of the results of this study, IOM (2011) (Section 4 and Appendix B) noted that about 1 % of subjects with a serum 25(OH)D concentration above 50 nmol/L had osteomalacia, while less than half of the subjects with serum 25(OH)D concentrations below 40 or even 25 nmol/L had osteomalacia. IOM (2011) used this study to consider that a serum 25(OH)D concentration of 50 nmol/L provides coverage for at least 97.5% of the population. **The Panel notes** that some concerns with regard to limitations of the Priemel study have been raised, such as the histomorphometric threshold used to define osteomalacia and the validity of *post mortem* 25(OH)D measurements (Aspray and Francis, 2013). However, the Panel considers that the threshold of OV/BV $\geq$ 2% used to define osteomalacia by Priemel et al. (2010) is a conservative approach. The Panel also notes that no studies are available showing whether post-mortem 25(OH)D measurements are valid.

Lamberg-Allardt et al. (2013) referred to the conclusion of IOM (2011) regarding osteomalacia and stated that no additional reduction in the risk of osteomalacia is to be expected at serum 25(OH)D concentrations above 50 nmol/L. Newberry et al. (2014) did not address the relationship between 25(OH)D and osteomalacia beyond the report by IOM (2011). SACN (2015) considered two cross-sectional studies (Preece et al., 1975; Gifre et al., 2011) as well as case reports on patients with osteomalacia from early 1940s to 2013 and concluded that evidence on vitamin D and osteomalacia is limited and, drawn mainly from case reports, that there is no clear serum 25(OH)D threshold concentration below which the risk of osteomalacia is increased, but noted that mean concentrations (in patients) were below about 20 nmol/L in all the studies considered. The Panel did not retrieve any additional pertinent primary study published from 2010 onwards.

The Panel notes that no recently published relevant data from RCTs or prospective observational studies on the association between serum 25(OH)D concentration and ostemalacia are available.

The Panel takes into account the findings by SACN (2015), based mainly on case-reports and two cross-sectional studies in patients with overt osteomalacia at mean serum 25(OH)D concentrations below about 20 nmol/L. Based on the limited evidence available (Priemel et al., 2010) and in line

with the conclusion of IOM (2011), the Panel considers that the risk of vitamin D-deficiency osteomalacia appears to be small with serum 25(OH)D concentrations at or above 50 nmol/L.

5.1.2.1.3. Fracture risk

IOM (2011) (Section 4 and Appendix B) reported that there was a wide variation in serum 25(OH)D concentrations below which fracture risk may be increased and that this was observed for serum 25(OH)D concentrations between 30 and 70 nmol/L.

Lamberg-Allardt et al. (2013) based their conclusions about risk of fractures in older adults on three systematic reviews (Avenell et al., 2009; Chung et al., 2009; Vestergaard et al., 2011). The overall conclusion in the NNR 2012 is that intervention with vitamin D alone has not been proven effective in preventing fractures in older adults, while the association of risk of fractures with serum 25(OH)D concentration was not specifically addressed. Newberry et al. (2014) did not identify any new RCTs that assessed the effect of interventions of vitamin D alone on fracture risk. They reported on six new observational studies that assessed the association between serum 25(OH)D and fracture risk (Cauley et al., 2011; Barbour et al., 2012; Barrett-Connor et al., 2012; de Boer et al., 2012; Holvik et al., 2013; Looker, 2013) and concluded that results were inconsistent among them. SACN (2015) additionally reported that evidence from five studies (Cauley et al., 2010; Cauley et al., 2011; Nakamura et al., 2011; Barbour et al., 2012; Rouzi et al., 2012) is mixed. SACN (2015) also considered studies (intervention and cohorts studies, systematic review of observational studies) about prevention of stress fractures in younger adults (less than 50 years) that were military personnel. Such a population was not considered by the Panel in this section (with the aim of setting DRVs for vitamin D for the general population).

The Panel retrieved 15 relevant prospective observational studies in non-institutionalised adults (but no RCTs), reporting on fractures in relation to 25(OH)D concentrations and that were published after the report by IOM (2011). In the following section, the *15 prospective observational studies* are described individually. The results are then summarized, and an *overall conclusion on fracture risk.*

Prospective observational studies

In a case-cohort study in men aged 65 years and older**,** Cauley et al. (2010) followed 436 men with incident non-spine fractures, including 81 hip fractures, and a random subcohort of 1,608 men over an average of 5.3 years. The mean baseline total 25(OH)D concentration was 61.5 ± 19.5 nmol/L in non-spine fracture subjects, 53.8 ± 19.8 nmol/L in hip fracture subjects and 63.0 ± 19.5 nmol/L in controls (non-spine fracture subjects versus non-patients, $p = 0.14$; hip fracture subjects versus controls, $p < 0.0001$). Serum 25(OH)D concentrations were unrelated to non-spine fractures. Compared with men in the top quartile of total 25(OH)D concentration (≥ 70 nmol/L), the hazard ratio (HR) of hip fracture was 2.36 (95% CI 1.08–5.15) for men in the lowest quartile (< 50 nmol/L) ($p = 0.009$ for trend), after adjustments for potential confounders[17]. The results were not always statistically significant when other additional adjustments were considered[18]. **The Panel notes** that, in these older men, serum 25(OH)D concentrations < 50 nmol/L (lowest quartile) were associated with an increased risk for hip, but not for non-spine fractures.

In a five year calcium supplementation study in Australia, Bolland et al. (2010) (Sections 5.1.1.1.1. and 5.1.1.1.4.1.) examined the association between baseline serum 25(OH)D concentration and multiple health outcomes in 1,471 community dwelling women (mean age 74 years). Fifty percent of women had a seasonally adjusted 25(OH)D concentration < 50 nmol/L. After adjustments for

[17] Age, race, clinic, season of blood draw, physical activity, weight, and height.
[18] Percent of body fat, or health status, or neuromuscular measures (unable to complete chair stand or narrow walk, grip strength), or hip BMD, or falls.

potential confounders (including treatment allocation to calcium or placebo), women with a seasonally adjusted baseline 25(OH)D concentration < 50 nmol/L were not at increased risk of fracture (hip, vertebral, distal forearm, osteoporotic), compared with those with 25(OH)D concentrations ≥ 50 nmol/L, and both groups did not show any difference in change in bone density (lumbar spine, total femur, total body). **The Panel notes** that this study of community-dwelling older women with a seasonally adjusted 25(OH)D concentration < 50 nmol/L compared with those with 25(OH)D concentrations ≥ 50 nmol/L showed no increased risk of fractures over a five year period.

In a nested case-control study in the USA in 400 white, 381 black, 193 Hispanic, 113 Asian and 46 Native American women (aged 50–79 years), Cauley et al. (2011) evaluated the incidence of fractures (all types) over an average of 8.6 years. In multivariable models, compared with concentrations < 50 nmol/L, higher baseline 25(OH)D concentrations ≥ 75 nmol/L were associated with a lower risk of fracture in white women (for 50 to < 75 nmol/L, odds ratio (OR): 0.82; 95% CI: 0.58-1.16; for ≥ 75 nmol/L: OR: 0.56; 95% CI: 0.35–0.90, p trend = 0.02). In contrast, higher 25(OH)D (≥ 50 nmol/L) compared with levels < 50 nmol/L were associated with a higher risk of fracture in black women (OR: 1.45; 95% CI: 1.06–1.98, p trend = 0.043), after adjustment for potential confounders. In Asian women, the OR for fracture at higher 25(OH)D concentrations (≥ 75 nmol/L) compared with 25(OH)D < 50 nmol/L, was 2.78 (95% CI: 0.99–7.80, p trend = 0.04). There was no association between 25(OH)D and fracture in Hispanic or Native American women. **The Panel notes** that, in this study, associations between 25(OH)D and fracture by race/ethnicity were divergent and that serum 25(OH)D were associated with significantly lower fracture risk in white women with baseline concentrations ≥ 75 nmol/L, but a higher fracture risk in black women with baseline concentrations ≥ 50 nmol/L.

In a cohort study, Nakamura et al. (2011) followed-up 773 community-dwelling Japanese women aged 69 years and older, for six years. Mean serum 25(OH)D concentration was 60.0 ± 17.6 nmol/L and mean calcium intake was 586 ± 259 mg/day. The adjusted HRs of limb and vertebral fracture for the first quartile (< 47.7 nmol/L) and the third quartile (59.2–70.9 nmol/L) of baseline serum 25(OH)D, 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[19]. The pooled adjusted HR was 0.42 (95% CI, 0.18–0.99) when the incidence in the fourth quartile (≥ 71.0 nmol/L) was compared to the other three quartiles combined (< 71.0 nmol/L). **The Panel notes** that, in this study in Japanese women with rather low calcium intake, risk for limb and vertebral fracture was higher at baseline serum 25(OH)D concentrations < 71 nmol/L (quartiles Q1–Q3).

In a cohort study, Robinson-Cohen et al. (2011) followed-up 2,294 U.S Caucasian and African American men and women (mean age: 74 years) for a median duration of 13 years. Baseline serum 25(OH)D was below 37.5 nmol/L for 382 participants. After adjustments for potential confounders, serum 25(OH)D concentrations less than 37.5 nmol/L were associated with a 61% greater risk of hip fracture (95% CI: 12–132%). **The Panel notes** that this study in both Caucasian and African American subjects indicated a greater risk for hip fractures at baseline serum 25(OH)D concentration < 38 nmol/L.

In a cohort study in Danish women (median age: 51 years) followed-up for 16 years (assessment after 10 years of follow-up) and with a mean baseline plasma 25(OH)D of about 65 nmol/L (Section 5.1.1.1.1), Rejnmark et al. (2011) also examined the risk of (all) fractures according to plasma 25(OH)D (below 50 nmol/L, at 50–80 nmol/L, and above 80 nmol/L) and tertiles of PTH concentrations. Plasma 25(OH)D concentrations *per se* were not associated with the risk of any fracture. High PTH concentrations (> 4.5 pmol/L) were associated with an increased fracture risk at 25(OH)D concentrations < 50 nmol/L (HR_{adj} = 1,71, 95% CI 1.1–2.66, p < 0.01) and at 25(OH)D concentrations 50–80 nmol/L (HR_{adj} = 1,60, 95% CI 1.07–2.37, p < 0.02). **The Panel notes** that this

[19] Fracture risk in the second quartile was not statistically different from the one in fourth quartile.

study in women indicated that baseline plasma 25(OH)D concentrations *per se* were not associated with fracture risk, but were related to fracture risk at concentrations < 80 nmol/L at high PTH concentrations. Thus, the relationship between 25(OH)D concentration and fracture risk was shown to depend on PTH.

In a cohort study in mobile community-dwelling Chinese men aged at least 65 years whose mean baseline 25(OH)D was about 78 ± 20 nmol/L (Section 5.1.1.1.1), Chan et al. (2011) also found, in multivariate regression analyses, no association between baseline serum 25(OH)D concentration (continuous variable or over quartiles of < 63 nmol/L up to > 91 nmol/L) and the four-year risk of non-vertebral or hip fractures. **The Panel notes** that this study in men with a mean serum 25(OH)D concentration of about 78 nmol/L found no association between baseline serum 25(OH)D concentrations and risk of non-vertebral or hip fractures.

In a cohort study with a median follow-up time of 6.4 years in U.S. community-dwelling white and black men and women aged ≥ 70 years (Section 5.1.1.1.1), Barbour et al. (2012) also investigated whether increasing serum 25(OH)D and decreasing PTH concentrations are associated with decreased risk of hip and any non-spine fracture, assessed every six months after year 2 ('baseline'). In multivariate analyses, there was no significant association between the risk of hip fracture and 25(OH)D concentration assessed as quartiles (≤ 44.5 nmol/L, 44.5–60.9 nmol/L, 60.9–79.9 nmol/L, compared to > 79.9 nmol/L). **The Panel notes** that this study in older subjects found no evidence of an association between baseline serum 25(OH)D concentrations ranging from < 45 nmol/L to ≥ 80 nmol/L (extreme quartiles) and any non-spine fractures.

In a case-cohort study in older men (mean age: 74 years) in the U.S.A., of which one quarter had 25(OH)D concentrations < 50 nmol/L with a mean of 38.8 nmol/L, Barrett-Connor et al. (2012) (Section 5.1.1.1.1) also tested the hypothesis that combinations of low 25(OH)D (< 50 nmol/L), low SH, and high SHBG would have a synergistic effect on non-spine fracture risk. Compared to men with 25(OH)D > 50 nmol/L, BioT > 163 ng/dL, BioE > 11 pg/mL, SHBG < 59 nmol/L, multivariate analyses showed no significant association between risk for incident non-spine and low 25(OH)D (< 50 nmol/L) in isolation, or low BioE and/or high SHBG in isolation. The multivariate-adjusted HR (95% CI) was 1.6 (1.1–2.5) for low BioE/high SHBG plus low 25(OH)D. Fracture risk for men with isolated low serum 25(OH)D, or those with low BioT with 25(OH)D > 50 nmol/L, did not differ from risk for men without low serum 25(OH)D or SH/SHBG abnormality. Significantly higher fracture risk was detected in the men with low BioE and/or high SHBG concurrent with a low 25(OH)D (adjusted HR, 95% CI: 1.62, 1.05–2.51). **The Panel notes** that, in these older men, the fracture risk associated with baseline serum 25(OHD) concentrations < 50 nmol/L (lowest quartile, mean 38.8 nmol/L) was observed only in the presence of low BioE or high SHBG, whereas 25(OH)D concentration < 50 nmol/L in isolation was not associated with fracture risk.

In a prospective cohort study, Rouzi et al. (2012) followed a cohort of 707 healthy Saudi postmenopausal women (mean age ± SD: 61.3 ± 7.2 years) for a mean ± SD of 5.2 ± 1.3 years. Their mean baseline serum 25(OH)D concentration was about 34 nmol/L. In multivariate logistic regression, besides physical activity score, age, hand-grip strength, BMD total hip, past year history of falls, baseline serum 25(OH)D concentration and dietary calcium intake in the lowest quartiles were identified as independent predictors of risk of all osteoporosis-related fractures. For the lowest quartile (Q1) serum 25(OH)D (≤ 17.9 nmol/L) vs higher values, relative risk (RR) was 1.63 (95% CI: 1.06–2.51, $p < 0.027$) and for dietary calcium intake in Q1 (≤ 391 mg/day) vs higher values, RR was 1.66 (95% CI: 1.08–2.53, $p < 0.020$). **The Panel notes** that this study in postmenopausal women indicated an increase in the risk for osteoporosis-related fractures at baseline serum 25(OH)D concentrations ≤ 17.9 nmol/L (lowest quartile).

In a pooled US cohort of 4,749 men and women aged 65 years and older from two surveys, Looker (2013) found that baseline serum 25(OH)D concentration was a significant linear predictor of risk of major osteoporotic fracture (hip, spine, radius, and humerus) and significant quadratic predictor

of hip fracture in the total sample and among those with less than 10 years of follow-up. It was not related to risk of either fracture type among those with 10 years of follow-up or more. After adjustments for potential confounders, fracture risk was significantly increased for serum 25(OH)D concentration < 30 nmol/L (major osteoporotic fracture RR: 2.09; 95% CI: 1.32–3.32; hip fracture RR: 2.63; 95% CI: 1.60–4.32), compared to serum 25(OH)D ≥ 30 nmol/L. Using other cut-off values, risk for either fracture outcome among those with serum 25(OH)D concentration between 30 and 49 nmol/L and 50 and 74 nmol/L did not differ from that seen in those with serum 25(OH)D ≥ 75 nmol/L, whereas the risk for either fracture was again significantly higher for those with serum 25(OH)D < 30 nmol/L. **The Panel notes** that this study in older subjects indicated an increase in the risk for fractures (major osteoporotic or hip only) at baseline serum 25(OH)D concentrations < 30 nmol/L.

Using a stratified case-cohort design in 21,774 men and women (65–79 years) who attended four community-based health studies in Norway with a maximum follow-up of 10.7 years, Holvik et al. (2013) found an inverse association between 25(OH)D concentration and risk of hip fracture. After adjustments for potential confounders, in the fully adjusted model, only subjects with 25(OH)D concentration in the lowest quartile (< 42.2 nmol/L) had a 34% (95% CI 5–70 %) increased risk of hip fracture compared with the highest quartile (≥ 67.9 nmol/L). After adjustment for age, gender, study centre and BMI, the association was statistically significant in men (HR 1.65; 95% CI: 1.04-2.61), but not in women, while the association was not statistically significant in either sexes in the fully adjusted model (including also month of blood sample). **The Panel notes** that, in this study in older subjects, an increased risk of hip fracture with baseline 25(OH)D concentrations < 42 nmol/L (lowest quartile) was observed, when compared to 25(OH)D concentrations ≥ 68 nmol/L (highest quartile).

In a population-based, prospective cohort study in Australia, Bleicher et al. (2014) followed 1,662 community-dwelling men (70-97 years) for a mean of 4.3 years (mean baseline 25(OH)D: about 56 nmol/L). In multivariate analyses[20], the risk of incident fractures was greatest only in men with baseline 25(OH)D concentrations in the lowest quintile (25(OH)D ≤ 36 nmol/L; mean 28.1 ± 6.6 nmol/L; HR: 3.5; 95% CI: 1.7–7.0) and in men in the highest quintile (25(OH)D > 72 nmol/L; HR: 2.7; 95% CI: 1.3–5.4), compared with men in the fourth quintile (25(OH)D ≥ 60 to ≤ 72 nmol/L). The difference in risk in quintiles 2 and 3 compared to 4 generally remained not statistically significant after additional adjustments[21] or a sensitivity analysis. **The Panel notes** that this study in older men indicated an increased risk for fractures in men at baseline serum 25(OH)D concentration < 36 nmol/L and > 72 nmol/L (lowest and highest quintiles).

In a prospective study of 5,764, both frail and healthy, men and women, aged 66–96 years, based on a representative sample of the population of Reykjavik, Iceland, HRs of incident hip fractures were determined according to serum concentrations of 25(OH)D at baseline (Steingrimsdottir et al., 2014). Mean follow-up was 5.4 years. Compared with serum 25(OH)D of 50–75 nmol/L, HRs for hip fractures were 2.08 (95% CI 1.51–2.87) for serum 25(OH)D < 30 nmol/L in the fully-adjusted model including physical activity. No difference in risk was associated with 30–50 nmol/L or ≥ 75 nmol/L in either model compared with the reference. This was also true when analysing men and women separately. **The Panel notes** that, in this study in older subjects, at baseline 25(OH)D concentrations of < 30 nmol/L, the risk for hip fractures increased, whereas no difference in the risk was observed over the range above 30 to 75 nmol/L.

In a U.S. prospective cohort study in 922 women during the menopausal transition and with an average follow-up of 9.5 years, Cauley et al. (2015) (Section 5.1.1.1.1.) determined if higher

[20] Adjusted for age, country of birth, BMI, physical activity, season of blood draw, previous low‐trauma fracture after age 50 years, calcium supplement, and vitamin D supplement.
[21] Additional adjustments for falls or BMD or neuromuscular measures (chair stands and narrow walk test) or serum $1,25(OH)_2D$ or multivariate model excluding subjects taking vitamin D supplements.

baseline 25(OH)D concentration is associated with lower fracture risk. The mean 25(OH)D concentration was 54.5 nmol/L; 43% of the women had 25(OH)D concentrations < 50 nmol/L. There was no significant association between serum 25(OH)D and traumatic fractures. However, in multivariable adjusted hazards models, the HR for non-traumatic fractures was 0.72 (95% CI: 0.54-0.95) for each 25 nmol/L increase in 25(OH)D, and was 0.54 (95% CI: 0.32–0.89) when comparing women whose 25(OH)D concentration was ≥ 50 vs < 50 nmol/L. **The Panel notes** that, in this study, serum 25(OH)D concentrations < 50 nmol/L were associated with an increased risk for non-traumatic fracture in mid-life women.

Conclusions on fracture risk in adults

Among the 15 recent prospective observational studies identified, most of which were in older non-institutionalised adults, the Panel notes the heterogeneity of observational study designs, populations and fracture sites investigated and considers that the relationship of serum 25(OH)D concentration and fracture risk may be confounded by a variety of factors (see Section 5.1.1.1.1). Furthermore, observational studies mostly used single measurements of 25(OH)D concentration, thus possible long-term changes in 25(OH)D concentration were not considered in the analyses of the relationship with fracture risk.

An increased risk of fractures was seen at baseline 25(OH)D concentrations < 18 nmol/L (Rouzi et al., 2012) (lowest quartile), < 30 nmol/L (Looker, 2013; Steingrimsdottir et al., 2014), < 36 nmol/L (Bleicher et al., 2014) (lowest quintile), < 38 nmol/L (Robinson-Cohen et al., 2011), < 42 nmol/L (Holvik et al., 2013) (lowest quartile), < 50 nmol/L ((Cauley et al., 2015); lowest quartile in (Cauley et al., 2010), lowest quartile and only in case of low sex steroid concentrations for (Barrett-Connor et al., 2012)), and < 71 nmol/L (Nakamura et al., 2011) (quartiles Q1–Q3). One study observed a significant negative relationship between PTH concentration and fracture risk at serum 25(OH)D concentrations < 50–80 nmol/L (Rejnmark et al., 2011). An increased fracture risk was also reported at 25(OH)D concentrations > 72 nmol/L (Bleicher et al., 2014) (highest quintile), > 50 nmol/L in black women and > 75 nmol/L in Asian (non statistically significant) women but a lower fracture risk at 25(OH)D < 75 nmol/L in white women (statistically significant) (Cauley et al., 2011). However, three studies found no difference in fracture risk between baseline serum 25(OH)D concentrations in the lowest quartile (< 45 nmol/L, (Barbour et al., 2012); < 50 nmol/L (Bolland et al., 2010); < 63 nmol/L, (Chan et al., 2011)) and higher concentrations.

The Panel notes that 9 out of 15 observational studies reported an increased risk for fractures that was associated with baseline 25(OH)D concentrations between < 18 nmol/L and < 50 nmol/L in non-institutionalised adult populations (Rouzi et al., 2012; Looker, 2013; Steingrimsdottir et al., 2014) (Barrett-Connor et al., 2012; Holvik et al., 2013; Bleicher et al., 2014; Cauley et al., 2015) (Cauley et al., 2010; Robinson-Cohen et al., 2011). One study observed a significant negative relationship between PTH concentration and fracture risk at serum 25(OH)D concentrations < 80 nmol/L (Rejnmark et al., 2011) and, in one study in Japanese women (with low calcium intake), an increased fracture risk was reported at 25(OH)D concentration < 71 nmol/L (Nakamura et al., 2011).

In contrast, an increased fracture risk was observed at ≥ 50 to ≥ 75 nmol/L in two studies ((Cauley et al., 2011), only in African American (significant result) and Asian (non-significant result) women, respectively; (Bleicher et al., 2014)), but not in others ((Cauley et al., 2011) in white women, (Chan et al., 2011; Barbour et al., 2012; Looker, 2013)).

The Panel notes the conclusions by IOM (2011) on a wide variation in serum 25(OH)D concentration associated with an increased fracture risk. **Taking into account also the observational studies published thereafter, the Panel considers that, overall,** the majority of studies indicate an increased fracture risk associated with 25(OH)D concentrations of < 18 nmol/L to < 50 nmol/L in non-institutionalised adults.

5.1.2.1.4. Muscle strength/function and physical performance

IOM (2011) (Section 4 and Appendix B) considered physical performance and falls as independent health outcomes, but because of the joint consideration of these outcomes in the literature, the available evidence was considered together. IOM (2011) reported some support, mainly from observational studies, for an association between 25(OH)D concentrations and physical performance, but concluded that high-quality observational evidence from larger cohort studies was lacking (Section 4.1.1).

Lamberg-Allardt et al. (2013) identified two systematic reviews with meta-analyses of RCTs on vitamin D and muscle strength in older subjects (Muir and Montero-Odasso, 2011; Stockton et al., 2011). Based on a meta-analysis of 17 RCTs (n = 5,072, mean age 60 years in most studies), Stockton et al. (2011) concluded that vitamin D supplementation does not have an effect on muscle strength in adults with mean baseline serum 25(OH)D concentrations ≥ 25 nmol/L, and that two RCTs (in patients) demonstrate an increase in hip muscle strength in adults with serum 25(OH)D concentrations < 25 nmol/L. The systematic review on 13 RCTs (n = 2,268) by Muir and Montero-Odasso (2011) concluded that vitamin D doses of 20–25 µg/day showed beneficial effects on balance and muscle strength in older adults (≥ 60 years of age). Mean baseline serum 25(OH)D concentrations were about 25-65 nmol/L in 12 RCTs that provided the information (mean baseline of 25–50 nmol/L in 10 of these RCTs). The Panel notes that only three references among the studies considered in these two systematic reviews were published in 2010 or afterwards, and that seven RCTs were in common in both systematic reviews.

Newberry et al. (2014) identified two new RCTs in older adults that examined the effects of one year of vitamin D supplementation with calcium on muscle strength or function (Pfeifer et al., 2009; Zhu et al., 2010). Newberry et al. (2014) also identified five prospective cohort studies on the association between serum 25(OH)D concentrations and muscle strength, muscle function or physical performance (Dam et al., 2009; Scott et al., 2010; Michael et al., 2011; Houston et al., 2012; Menant et al., 2012). Newberry et al. (2014) concluded that the associations between serum 25(OH)D concentrations and muscle strength, muscle function or physical performance in postmenopausal women or older men were inconsistent.

SACN (2015) considered three systematic reviews with meta-analyses of RCTs (two already mentioned above (Muir and Montero-Odasso, 2011; Stockton et al., 2011) and another one (Beaudart et al., 2014)[22] on 30 RCTs (n = 5,615). These systematic reviews reported a beneficial effect of vitamin D supplementation on muscle strength and function in adults aged > 50 years with mean baseline serum 25(OH)D concentrations of 24–66 nmol/L (Muir and Montero-Odasso, 2011), < 30 nmol/L (Beaudart et al., 2014), and < 25 nmol/L (patients (Stockton et al., 2011)). The Panel notes that 14 RCTs out of the 30 RCTs included in (Beaudart et al., 2014) were published in 2010 or afterwards[23], and 8 or 11 references were in common with the systematic review by (Muir and Montero-Odasso, 2011) or by (Stockton et al., 2011), respectively. SACN identified three subsequent RCTs (Lips et al., 2010; Knutsen et al., 2014; Pirotta et al., 2015) and seven cohort studies (Bolland et al., 2010; Scott et al., 2010; Houston et al., 2011; Michael et al., 2011; Chan et al., 2012; Houston et al., 2012; Menant et al., 2012), which provided mixed results, and also noted that, in most of the cohort studies, cut-offs were predefined.

The Panel considered pertinent primary studies from 2010 onwards mostly on healthy adults and, when excluding studies in populations with resistance training, retrieved 14 intervention and prospective observational studies, reporting on muscle strength or function, physical performance or related outcomes (e.g. postural stability, muscle power, mobility), in relation to 25(OH)D

[22] Some studies also on vitamin D metabolites/analogues were considered in these systematic reviews.
[23] Some of these studies are described below. Others were undertaken e.g. with vitamin D metabolite or based on a frequency of supplementation (e.g. once per three months) that did not match the inclusion criteria of the Panel (Section 5.1.).

concentrations. In the following section, the *eight intervention studies* and then the *six prospective observational* studies are described individually. The results are then summarized, and an *overall conclusion on muscle strength/function and physical performance* is provided.

RCTs with vitamin D supplementation

In a 16-week double-blind multicentre RCT in North America and Europe, Lips et al. (2010) studied the effects of a dose of 210 µg vitamin D_3 per week (~ 30 µg/day) or a placebo on **postural stability,** measured as postural body sway, and **physical performance,** measured as short physical performance battery (SPPB), in 246 older subjects (age 70 years and older). Baseline serum 25(OH)D concentrations were between 15 and 50 nmol/L. Mean serum 25(OH)D concentrations increased significantly from 35 to 65 nmol/L ($p < 0.001$) in subjects receiving 210 µg/week, with no change in the placebo group. No differences in postural stability or physical performance were observed between groups at the end of the study. In a post-hoc analysis of a subgroup of patients with elevated sway at baseline, supplementation with vitamin D_3 significantly reduced sway. **The Panel notes** that this study in older subjects with weekly vitamin D_3 supplementation, which increased their mean serum 25(OH)D concentration from 35 to 65 nmol/L, found no effect on postural stability or physical performance compared with placebo. The Panel also notes that the study found an increased postural stability in those with elevated body sway at baseline.

In a six-month double-blind RCT in the Netherlands, Janssen et al. (2010) compared the effects of a daily supplementation of 10 µg vitamin D_3 and 500 mg calcium with a placebo + 500 mg calcium supplementation only, on **muscle strength** (knee extension or handgrip strength)**, power** (leg extension power) **and mobility** (Timed Up And Go (TUAG) test and Modified Cooper test[24]) in 70 female geriatric outpatients. Most participants lived in residential homes, all were above 65 years of age with baseline serum 25(OH)D concentrations between 20 and 50 nmol/L (mean baseline of 33-34 nmol/L among groups). At six months, a significant difference in mean serum 25(OH)D (77.2 vs 41.6 nmol/L, $p < 0.001$) and $1,25(OH)_2D$ concentrations (94.1 vs 67.5 pmol/L, $p < 0.001$) was found between the two groups, but no differences in muscle strength, power or mobility. **The Panel notes** that, in this study, older subjects supplemented daily with vitamin D_3 and calcium for six months, compared with calcium alone, increased their mean serum 25(OH)D from 33 to 77 nmol/L compared with increases from 34 to 42 nmol/L in the placebo + calcium group, and that no effect on muscle strength, power or mobility was measured.

In a one-year population-based double-blind RCT in Australia, Zhu et al. (2010) assessed the effects of a daily 25 µg vitamin D_2 supplement or placebo (both groups receiving 1 g calcium/day) on **muscle strength** in different muscle groups **and mobility using** the TUAG test in 302 older community-dwelling women aged 70-90 years. Mean baseline serum 25(OH)D was 44 ± 10.5 nmol/L (with 66% of subjects with 25(OH)D concentration lower than 50 nmol/L). In the vitamin D and calcium group after one year, 25(OH)D concentration increased to 60 ± 14 nmol/L (with 80% of subjects achieving a serum 25(OH)D concentration higher than 50 nmol/L). For hip extensor and adductor strength and TUAG, but not for other muscle groups, a significant interaction between treatment group and baseline values of 25(OH)D was noted. Only in those subjects in the lowest tertile of baseline hip extensor and adductor strength and TUAG test, vitamin D and calcium supplementation improved muscle strength and TUAG test more compared with calcium supplementation alone. Baseline 25(OH)D concentrations did not influence subject's response to supplementation with regard to muscle strength and mobility. **The Panel notes** that this study in older women supplemented daily with vitamin D_2 together with calcium for 12 months increased mean serum 25(OH)D concentration from 44 to 60 nmol/L, compared with calcium alone, and that increased muscle strength and mobility were found only in those who were the weakest and slowest at baseline.

[24] The Modified Cooper test is used as a measurement of overall mobility.

In a six-month double-blind, randomised exploratory clinical trial in the U.S.A., Lagari et al. (2013) investigated the effects of daily 10 or 50 µg vitamin D_3 supplementation on **physical performance and muscle strength**, in 86 community-dwelling subjects aged 65 to 95 years with a mean baseline serum 25(OH)D concentration of 82.5 nmol/L. Physical performance was assessed as a four-meter walk speed test to calculate gait speed, timed sit-to-stand test or chair stand test, single-leg balance test and gallon-jug test, and muscle strength was measured as handgrip test. A mean decrease in serum 25(OH)D concentration of 3 nmol/L in men (n = 6) and 8.5 nmol/L in women (n = 25) was observed in the 10 µg/day supplement group and a mean increase was observed in the 50 µg/day supplement group of 16 nmol/L in men (n = 9) and 13 nmol/L in women (n = 46). Overall, no significant changes in physical performance or muscle strength were found at the end of the intervention period. However, subjects with the slowest gait speed at baseline improved their ability to do chair-stand tests after vitamin D supplementation, after adjustments for potential confounders. **The Panel** notes that, in this study in older subjects, two daily doses of vitamin D_3 supplementation for six months decreased (- 3 to - 8.5 nmol/L) or increased serum 25(OH)D concentrations (+ 13 to + 16 nmol/L) from a mean baseline of 82.5 nmol/L, and that no effect of dose on physical performance or muscle strength was measured. The study showed that subjects with the slowest gait speed at baseline showed an improvement in one of the physical performance tests.

In a 12-week RCT in the UK in 25 young athletes (mean age 21 years) receiving either placebo, 500 µg or 1,000 µg/week vitamin D_3 (~ 71 µg/day and 142 µg/day), Close et al. (2013a) measured serum 25(OH)D concentration and **muscle function** (bench press and leg press and vertical jump height) before, at 6 and at 12 weeks post-supplementation. Baseline mean serum 25(OH)D concentration was 51 ± 24 nmol/L, with 57% of subjects below 50 nmol/L. Following 6 and 12 weeks supplementation, serum 25(OH)D concentrations increased above 50 nmol/L in all participants (mean in each group: about 85–90 nmol/L (values read on figure)). In contrast, 25(OH)D concentration in the placebo group decreased at six and 12 weeks to 37 ± 18 and 41 ± 22 nmol/L, respectively. None of the muscle function parameters in these young athletes was significantly affected by an increase of serum 25(OH)D concentration. **The Panel notes** that, in younger subjects, weekly doses of vitamin D_3 supplementation for 12 weeks increased their serum 25(OH)D concentration above 50 nmol/L, and that this study found no effect on muscle function compared with placebo.

In a parallel group double-blind RCT by Wood et al. (2014), healthy postmenopausal women from North East Scotland aged 60–70 years, were assigned to daily vitamin D_3 of 10 µg (n = 102), 25 µg (n = 101) or matching placebo (n = 102) for one year. **Grip strength** (primary outcome), diet, physical activity and ultraviolet B radiation exposure were measured bimonthly, as were serum 25(OH)D, adjusted calcium and phosphate. Mean (SD) serum 25(OH)D concentrations at baseline were 34.3 (14.7) nmol/L, 33.9 (14.3) nmol/L and 32.4 (16.3) nmol/L in normal weight (BMI < 25 kg/m^2; n = 113), overweight (BMI 25–25.99 kg/m^2; n = 139) and obese (BMI ≥ 30 kg/m^2; n = 53) subjects, respectively. After one year of treatment with 10 and 25 µg of vitamin D, serum 25(OH)D concentration had increased between by 32-33 µmol/L and 38.8-48.1 nmol/L, respectively, among the various BMI groups. In contrast, the change in 25(OH)D in the placebo groups was between - 1.7 to - 6.6 µmol/L. **The Panel notes** that, in this study, two different daily doses of vitamin D_3 supplementation for one year increased mean serum 25(OH)D concentration, but had no effect on grip strength compared to placebo.

In a 16-week randomised, double-blind, placebo-controlled trial in Norway, Knutsen et al. (2014) compared the effects of a daily vitamin D_3 supplementation (10 or 25 µg vitamin D_3) or placebo on **muscle power and strength** measured as jump height and handgrip strength and chair-rising differences between pre- and post-intervention in adults from ethnic minority groups (n = 215) with a mean age of 37 years (range 18–50 years). Mean serum 25(OH)D_3 concentration increased from 27 to 52 nmol/L and from 27 to 43 nmol/L in the groups receiving 25 and 10 µg/day, respectively, with no changes in the placebo group. Vitamin D supplementation had no significant effect on muscle power or strength. **The Panel notes** that this 16-week study in younger adults from minority

ethnic groups with two daily supplemental doses of vitamin D_3 increased mean 25(OH)D concentration from 27 to 52 or 43 nmol/L with no significant effect on muscle power or muscle strength compared with placebo.

In a 10-week RCT in Australia, Pirotta et al. (2015) investigated the effects of a daily supplement (50 µg vitamin D_3 or a placebo) in 26 older adults (> 60 years) with baseline 25(OH)D concentrations between 25–60 nmol/L on neuroplasticity as the primary outcome and **muscle power and function** (mobility) measured as stair climbing power, gait (TUAG), dynamic balance (four square step test) as the secondary outcome. Mean serum 25(OH)D concentration increased from 46 to 81 nmol/L in the vitamin D supplemented group with no changes in the placebo group. No significant changes in any of the outcome measures were observed between the vitamin D supplemented and placebo groups at the end of the intervention period. **The Panel notes** that this was a relatively short intervention study and that it showed that daily vitamin D supplementation increased mean serum 25(OH)D concentration from 46 to 81 nmol/L with no effect on muscle power or function in older adults compared with placebo.

Prospective observational studies

In a cohort of 686 community-dwelling older adults (mean age 62 ± 7 years, 49% women) in Australia, Scott et al. (2010) investigated associations between serum 25(OH)D concentration and leg muscle **strength** and leg muscle **quality** (LMQ)[25] at baseline and at a mean follow-up of 2.6 ± 0.4 years. At baseline, 297 subjects had serum 25(OH)D concentration ≤ 50 nmol/L (mean ± SD of 37.1 ± 8.4 nmol/L), and 389 had serum 25(OH)D > 50 nmol/L (mean ± SD of 67.8 ± 13.4 nmol/L). After adjustments for potential confounders, baseline 25(OH)D concentration was positively associated with the change in leg muscle strength and LMQ over 2.6 years. **The Panel notes** that, in this study in older adults in which about 43% had baseline serum 25(OH)D below 50 nmol/L, baseline 25(OH)D concentration was positively associated with the change in leg muscle strength and LMQ.

In a five year calcium supplementation study in Australia, Bolland et al. (2010) (Section 5.1.1.1.1. and 5.1.1.1.3.) examined the association between baseline serum 25(OH)D concentration and multiple health outcomes in 1 471 community dwelling women (mean age 74 years). Fifty percent of women had a seasonally adjusted 25(OH)D concentration < 50 nmol/L. After adjustments for potential confounders (including treatment allocation to calcium or placebo), women with a seasonally adjusted baseline 25(OH)D concentration < 50 nmol/L and those with 25(OH)D concentrations ≥ 50 nmol/L did not show any difference in change in **grip strength**. **The Panel notes** that this study of community-dwelling older showed no difference in change in grip strength in women with a seasonally adjusted baseline 25(OH)D concentration < 50 nmol/L compared with those with 25(OH)D concentrations ≥ 50 nmol/L, over a five year period.

In a cohort of 534 US postmenopausal women (mean age: 70.3 ± 3.9 years, mainly Caucasian), Michael et al. (2011) evaluated the association between baseline serum 25(OH)D concentration (48.2 ± 21.4 nmol/L) and a **physical summary score** at baseline, at 1, 3 and 6 years. The physical summary score was derived from data on timed walk test, chair-stand test and grip strength. In the six years of follow-up, participants with baseline serum 25(OH)D concentration ≥ 75 nmol/L (but not those with 25(OH)D of 25–49 and 50–74 nmol/L) had significantly higher scores for physical performance compared with the reference category (< 25 nmol/L) after adjustments for potential confounders ($p < 0.001$). Physical performance declined over the follow-up period as a result of ageing, but higher baseline serum 25(OH)D concentration was not associated with a reduction in the decline in physical performance over the six-year period. **The Panel notes** that this study showed that higher baseline serum 25(OH)D concentrations (≥ 75 nmol/L) in older women were associated with higher physical performance at follow-up compared with baseline concentrations < 25 nmol/L, but were not associated with the age-related decline in physical performance over a six-year period.

[25] Leg muscle quality (LMQ) defined as the level of force produced per unit of muscle mass.

In community-dwelling men and women aged 77–100 years in four different US settings, Houston et al. (2011) examined the association between baseline serum 25(OH)D concentrations and **mobility disability** (difficulty walking half a mile or up 10 steps) and **activities of daily living (ADL) disability** measured at baseline and every six months over three years of follow-up (longitudinal analysis). Almost one-third (31%) of participants had serum 25(OH)D concentrations < 50 nmol/L at baseline. After adjustments for potential confounders, in participants free of mobility disability at baseline, participants with baseline serum 25(OH)D concentration < 50 nmol/L (but not participants with serum 25(OH)D of 50–74 nmol/L) were at greater risk of incident mobility disability over three years of follow-up (HR:1.56; 95% CI: 1.06–2.30), compared with those with serum 25(OH)D concentration ≥ 75 nmol/L. In participants free of ADL disability at baseline, there was no association between baseline serum 25(OH)D concentration and risk of ADL disability. **The Panel notes** that, in this study in older community-dwelling adults, participants with baseline serum 25(OH)D concentrations < 50 nmol/L had a greater risk of incident mobility disability (but not of ADL disability) after three years of follow-up compared with those with serum 25(OH)D ≥ 75 nmol/L.

In a cohort of 2,641 men and women (age 71–80 years), 38% African American, in the USA, Houston et al. (2012) investigated associations between serum 25(OH)D measured at baseline and **physical performance**, measured as SPPB and the second physical performance battery, **gait speed** (20-m or 400-m), and **muscle strength** (knee extensor strength and grip strength), measured at baseline and at two and four years follow-up. After full adjustments for potential confounders, longitudinal associations between baseline 25(OH)D concentration and physical performance at four-year follow-up showed that participants with serum 25(OH)D < 50 nmol/L (but not those with serum 25(OH)D of 50–74 nmol/L) had poorer physical performance than participants with 25(OH)D ≥ 75 nmol/L ($p < 0.01$ for both battery scores) and lower 400-m gait speed ($p < 0.001$). Baseline serum 25(OH)D was not associated with muscle strength at the four-year follow-up. Physical performance and gait speed declined over the four years of follow-up ($p < 0.0001$), and, except for SPPB, the rate of decline was not associated with baseline 25(OH)D concentration. **The Panel notes** that this study in older subjects showed a poorer physical performance at four years (but not muscle strength) in subjects with baseline serum 25(OH)D concentrations < 50 nmol/L compared with ≥ 75 nmol/L, but that serum 25(OH)D concentrations at baseline was not related to the age-related decline in physical performance and strength over a four year follow-up.

In a longitudinal analysis of a prospective cohort study in China of community dwelling men (n = 714; age > 65 years), Chan et al. (2012) analysed the association between baseline serum 25(OH)D concentrations and four-year **physical performance** measures (including as grip strength, 6-m walking speed, step length in a 6-m walk, time to complete five chair stands). Baseline mean ± SD serum 25(OH)D concentration was 77.9 ± 20.5 nmol/L with 94% of participants having a concentration of 50 nmol/L or greater. After adjustment for potential confounding factors, serum 25(OH)D levels were not associated with baseline or four-year change in physical performance measures. The Panel notes that this study in older community dwelling men with relative high baseline serum 25(OH)D concentration showed no association with physical performance after a four-year period.

Conclusions on muscle strength/function and physical performance in adults

The Panel notes the heterogeneity in the design of the seven RCTs with respect to age profile of subjects, dose and length of administration of vitamin D with or without calcium, and measures of muscle strength and physical performance or related outcomes. The Panel notes that five RCTs were carried out in older not-institutionalised subjects (Janssen et al., 2010; Lips et al., 2010; Zhu et al., 2010; Lagari et al., 2013; Pirotta et al., 2015).

The Panel notes that, in the **eight RCTs** with vitamin D supplementation (with or without calcium) between 10 weeks and one year, mean serum 25(OH)D concentrations increased from 27 nmol/L (Knutsen et al., 2014), 33 nmol/L (Janssen et al., 2010), about 32–34 nmol/L (Wood et al., 2014),

35 nmol/L (Lips et al., 2010), 44 nmol/L (Zhu et al., 2010), 46 nmol/L (Pirotta et al., 2015), 51 nmol/L (Close et al., 2013a), or 82.5 nmol/L (Lagari et al., 2013), up to 52 nmol/L, 77 nmol/L, about 82 nmol/L, 65 nmol/L, 60 nmol/L, 81 nmol/L, about 90 nmol/L, or about 98 nmol/L, respectively. These RCTs showed that increasing mean serum 25(OH)D concentrations from these baseline to final values by vitamin D supplementation did not result in a change in measures of physical performance or muscle strength/function.

The Panel notes that all six **prospective observational studies** identified on the association between baseline serum 25(OH)D concentration and muscle strength/physical performance were on older subjects, but otherwise were heterogeneous with respect to design, and that the studies may be confounded by a variety of factors (Sections 5.1.1.1.1. and 5.1.1.1.3). Furthermore, as for other health outcomes (Sections 5.1.1.1.1. and 5.1.1.1.3), observational studies used single measurements of 25(OH)D concentration, thus possible long-term changes in 25(OH)D concentration were not considered in the analyses of the relationship with muscle strength/physical performance.

In one study in older adults in which about 43 % had baseline serum 25(OH)D below 50 nmol/L, baseline 25(OH)D concentration was positively associated with the change in leg muscle strength and LMQ (Scott et al., 2010). Three other observational studies (Houston et al., 2011; Michael et al., 2011; Houston et al., 2012) used pre-defined cut-off concentration for serum 25(OH)D, of < 25 nmol/L (versus 25–49, 50–74 and ≥ 75 nmol/L) (Michael et al., 2011), or > 75 nmol/L (versus < 50 or 50–74 nmol/L) (Houston et al., 2011; Houston et al., 2012). Among these three studies, two studies showed a higher risk of mobility disability as well as poorer physical performance in men and women with baseline serum 25(OH)D concentrations below 50 nmol/L (versus ≥ 75 nmol/L) (Houston et al., 2011; Houston et al., 2012). A third study, in older women, showed a better physical performance at six-year follow-up with baseline serum 25(OH)D concentrations ≥ 75 nmol/L (versus < 25 nmol/L) (Michael et al., 2011). In contrast, one study showed no difference in change in muscle strength (grip strength) in women with a seasonally adjusted baseline 25(OH)D concentration < 50 nmol/L (pre-defined cut-off) compared with those with 25(OH)D concentrations ≥ 50 nmol/L (Bolland et al., 2010). Finally, one study showed no association between serum 25(OH)D (mean baseline: 78–94 nmol/L) and measures of physical performance (Chan et al., 2012). The Panel notes that the observational studies were inconsistent in their findings.

In its conclusion, the Panel took into account the conclusions by IOM (2011) on some (mainly observational) evidence supporting an association between serum 25(OH)D concentrations and physical performance and on the lack of large high-quality observational evidence, the conclusions of Lamberg-Allardt et al. (2013), Newberry et al. (2014) and SACN (2015). The Panel also took into account the identified studies published thereafter, and notes that the evidence is inconsistent. The Panel considers that, overall, the recent RCTs, all undertaken in populations with mean baseline serum 25(OH)D concentration of 27 nmol/L or higher, show no support for an association between serum 25(OH)D concentration and physical performance in older adults. Four of the six new prospective observational studies used pre-defined cut-off values for serum 25(OH)D concentration. The Panel considers that four out of six observational studies reported a positive association between baseline serum 25(OH)D and better muscle strength/quality, lower risk of mobility disability or of poorer physical performance at follow-up. **Overall, from the available evidence, the Panel considers** that no target value for serum 25(OH)D concentration with regard to muscle strength/function and physical performance can be derived.

5.1.2.1.5. Risk of falls and falling

A fall is defined as "the unintentional coming to rest on the ground, floor, or other lower level" and the number of falls in a population subgroup over a period of time can be recorded and results expressed as, e.g. the number of falls per person per observation time (incidence), the total number of falls or the number of subjects falling at least once (termed fallers) (EFSA NDA Panel, 2011)).

IOM (2011) (Section 4 and Appendix B) concluded that the greater part of RCTs found no effects of vitamin D with or without calcium on reduction in the risk for falls and that a number of the RCTs analysed falls rather than fallers. IOM (2011) also concluded that the observational studies (mostly cross-sectional) suggested an association between a higher serum 25(OH)D concentrations and a reduced risk of falls in older adults.

Lamberg-Allardt et al. (2013) based their conclusions on seven systematic reviews (Cranney et al., 2007; Chung et al., 2009; Kalyani et al., 2010; Michael et al., 2010; Murad et al., 2011; Cameron et al., 2012; Gillespie et al., 2012). Lamberg-Allardt et al. (2013) noted that the systematic reviews included many of the same studies, with some variation due to different inclusion and exclusion criteria and timeframe, and that the definition of 'falls' and 'falling' varied among trials. Lamberg-Allardt et al. (2013) concluded that there is a probable evidence that supplementation with vitamin D in combination with calcium is effective in preventing falls in older adults, especially in those with 'low' baseline serum 25(OH)D concentrations in both community dwelling and in nursing care facilities. The threshold for a 25(OH)D concentration below which the risk for falls or falling was increased was unclear.

Newberry et al. (2014) identified two RCTs, already cited in the IOM report, and that examined the effect of supplementation with vitamin D and calcium on the risk of falls/falling among older adults (Prince et al., 2008; Pfeifer et al., 2009), as well as one prospective cohort study (Menant et al., 2012) on serum 25(OH)D concentration and the risk of falls. Newberry et al. (2014) concluded that an association was seen between lower serum 25(OH)D concentrations and increased risk of falls.

SACN (2015) considered five systematic reviews and meta-analyses (Kalyani et al., 2010; Murad et al., 2011; Cameron et al., 2012; Gillespie et al., 2012; Bolland et al., 2014), one RCT (Sanders et al., 2010), one cohort study (Menant et al., 2012), and two genetic studies (Onder et al., 2008; Barr et al., 2010). The SACN concluded that the evidence on vitamin D and falls is mixed but, on balance, that the evidence is suggestive of beneficial effects of vitamin D supplementation in reducing fall risk in adults > 50 years with mean baseline serum 25(OH)D concentrations over a broad range of values (23–59, 24–28, 24–55, 23–82 nmol/L according to the systematic reviews considered).

In addition to the RCT by Wood et al. (2014) (Section 5.1.2.1.5.), the Panel identified one prospective observational study in non-institutionalised older adults published after the IOM report, that is described hereafter and followed by an *overall conclusion on risk of falls and falling*.

RCTs with vitamin D supplementation

In the double-blind RCT in healthy postmenopausal women from Scotland (60–70 years) assigned to daily vitamin D_3 of 10 µg (n = 102), 25 µg (n = 101) or matching placebo (n = 102) for one year (mean baseline serum 25(OH)D: about 32–34 nmol/L) (Section 5.1.2.1.5.), Wood et al. (2014) also measured falls bimonthly (secondary outcome) among the various BMI groups. **The Panel notes** that, in this study, two different daily doses of vitamin D_3 supplementation for one year increased mean serum 25(OH)D concentration, but had no effect on the number of 'ever fallen' falls compared to placebo.

Prospective observational study

In a cohort of 463 older community-dwelling men and women (54 %) (age 70–90 years) in Australia, Menant et al. (2012) studied the relationship between baseline serum 25(OH)D concentrations and falls monitored with monthly diaries and assessed at 12-months follow-up. At baseline, 21% of men and 44% of women had serum 25(OH)D concentrations ≤ 50 nmol/L. After adjustments for potential confounders, baseline serum 25(OH)D concentrations < 50 nmol/L (pre-defined cut-off) were associated with an increased rate of falls in men (incident rate ratio: 1.93; 95% CI : 1.19–3.15, p = 0.008), but not in women. **The Panel notes** that this study in older subjects showed that serum 25(OH)D concentrations < 50 nmol/L were associated with increased rate of

falls in men only. Furthermore, as for other health outcomes (Sections 5.1.1.1.1., 5.1.1.1.3 and 5.1.1.4.), this observational study used single measurements of 25(OH)D concentration, thus possible long-term changes in 25(OH)D concentration were not considered in the analyses of the relationship with rate of falls.

Conclusions on risk of falls and falling in adults

The Panel considered one RCT published after the IOM report, which showed that mean serum 25(OH)D concentrations increased after vitamin D_3 supplementation for one year, while this supplementation had no effect on the number of 'ever fallen' falls compared to placebo. The Panel considered one prospective observational study published after the IOM report. This study in older subjects showed that serum 25(OH)D concentrations < 50 nmol/L were associated with increased rate of falls in men only (Menant et al., 2012). The Panel considered the conclusion by IOM (2011), by SACN (2015), Newberry et al. (2014), Lamberg-Allardt et al. (2013), that took several systematic reviews (undertaken with different inclusion criteria) into account. The Panel notes that the evidence on serum 25(OH)D is inconsistent, but overall, is suggestive of beneficial effects of vitamin D in reduction of the risk of falling in older adults over a broad range of mean baseline serum 25(OH)D concentrations (23 to 82 nmol/L according to the systematic reviews considered in previous reports). **From the available evidence, the Panel concludes** that no target value for serum 25(OH)D concentration with regard to the risk of falls or falling can be derived.

5.1.2.1.6. Calcium absorption

Regarding the physiological role of $1,25(OH)_2D$ in the active transport regulation of calcium absorption in the intestine (Section 2.2.1) (EFSA NDA Panel, 2015a), the Panel considered it pertinent to review the possible relationship between 25(OH)D concentrations and calcium absorption to try to identify a possible threshold value for this relationship. Calcium absorption is usually measured as fractional calcium absorption for which the dual calcium isotopes technique is regarded as the gold standard (Heaney, 2000; IOM, 2011), whereas single isotope methods, which are considered more convenient to use, have also been developed (Lee et al., 2011).

IOM (2011) (Section 4 and Appendix B) considered both RCTs and cross-sectional studies in relation to vitamin D status and calcium absorption and concluded that fractional calcium absorption reaches a maximum at serum 25(OH)D concentrations between 30 and 50 nmol/L in adults, 'with no clear evidence of further benefit above 50 nmol/L'. The Panel notes that the IOM included the study by Need et al. (2008) in patients attending osteoporotic clinics, which found that 'low' vitamin D status does not reduce serum $1,25(OH)_2D$ concentration, and therefore calcium absorption, until the serum 25(OH)D concentrations falls to around 10 nmol/L and suggested this concentration below which the formation of $1,25(OH)_2D$ is compromised. The Panel notes that neither Lamberg-Allardt et al. (2013), nor Newberry et al. (2014) or SACN (2015) considered the relationship between serum 25(OH)D concentration and calcium absorption.

For studies post-dating the IOM report, the Panel identified several studies, including two RCTs (Shapses et al., 2013; Aloia et al., 2014) and one observational study (Shapses et al., 2012) using the *dual isotope technique* to measure fractional calcium absorption. The Panel also identified two RCTs (Gallagher et al., 2012; Gallagher et al., 2014) that used a *single isotope technique*. They were considered as supportive evidence by the Panel and are described individually below, followed by a summary of the results and an *overall conclusion on calcium absorption in adults*.

With regard to results obtained with the dual isotope technique, in a six-week placebo-controlled, double-blind RCT, Shapses et al. (2013) measured fractional calcium absorption in 83 postmenopausal women (mean age 57.8 ± 0.7 years, mean BMI of 30.2 ± 3.7 kg/m^2, mean baseline serum 25(OH)D concentration of 62.3 ± 14.3 nmol/L), during either a weight loss or

weight maintenance period. All women were given 1.2 g calcium/day and 10 µg vitamin D_3/day, and either weekly vitamin D_3 (375 µg) or a placebo, equivalent to a total supplementation of 63 µg/day and 10 µg/day, respectively, both sufficient to maintain calcium balance. The study showed that vitamin D supplementation increases fractional calcium absorption. **The Panel notes**, however, that no correlation was found between fractional calcium absorption and either serum 25(OH)D or $1,25(OH)_2D$ concentrations at baseline or after the intervention, in this study with mean baseline serum 25(OH)D concentration of 62.3 nmol/L.

In an eight-week placebo-controlled, double-blind RCT, Aloia et al. (2014) determined fractional calcium absorption in 71 healthy women (age 58.8 ± 4.9 years; mean BMI of the groups of 26.0-27.6 kg/m^2, and mean baseline serum 25(OH)D concentration of 63 ± 14 nmol/L, range: 30 to > 75 nmol/L), who were assigned to placebo, 20, 50, or 100 µg/day of vitamin D_3. After adjustment for potential confounders, there was a statistically significant linear relationship between an increase in 10-week calcium absorption and increasing vitamin D_3 doses ($R^2 = 0.41$, $p = 0.03$) and a marginally significant linear effect by10-week serum 25(OH)D concentration ($p = 0.05$, R^2 not reported). The changes (follow-up minus baseline) in serum 25(OH)D concentration and in calcium absorption were not significantly correlated. **The Panel notes** that no threshold value for serum 25(OH)D concentration in relation to calcium absorption was found in this study with final serum 25(OH)D concentrations between 40 and 130 nmol/L.

In a retrospective observational study, Shapses et al. (2012) examined the influence of body weight and hormonal and dietary factors on fractional calcium absorption in 229 adult women (age 54 ± 11 years, and BMI of 31.0 ± 7.0 kg/m^2). When categorised into tertiles of BMI, mean serum 25(OH)D concentrations were significantly lower (63 nmol/L) in the obese group (mean BMI 39.0 ± 10.4 kg/m^2) compared with the over- or normal weight groups (75 nmol/L) ($p < 0.05$), whereas mean $1,25(OH)_2D_3$ concentrations were similar. Fractional calcium absorption was significantly ($p < 0.05$) higher in obese women compared to non-obese women. After adjustment for multiple confounders, $1,25(OH)_2D_3$ was a significant predictor of fractional calcium absorption ($p = 0.042$), but not 25(OH)D. **The Panel notes** that no threshold value of 25(OH)D concentration in relation to fractional calcium absorption was found in this study.

With regard to results obtained with the single-isotope technique, in a one year double-blind RCT, Gallagher et al. (2012) measured calcium absorption, expressed as percentage of the actual dose per litre of plasma, at baseline and 12 months in 163 postmenopausal Caucasian women (age 57-90 years) with baseline serum 25(OH)D concentrations in the range of 12.5–50 nmol/L. Participants received one of the vitamin D_3 supplementation doses of 10, 20, 40, 60, 80, 100, or 120 µg/day or placebo and mean serum 25(OH)D increased from a mean value of 38 nmol/L at baseline (all subjects) to 112 nmol/L in subjects with the highest dose of vitamin D. Calcium absorption at 12 months was more related to 12-month serum 25(OH)D concentration ($R^2 = 0.51$, $p < 0.001$) than to dose ($R^2 = 0.48$, $p < 0.033$), after adjustments for potential confounders. There was however no evidence for a threshold value for a reduced calcium absorption in the 12-month serum 25(OH)D concentration range of 25–165 nmol/L (values read on figure). In another one-year double-blind RCT, Gallagher et al. (2014) measured calcium absorption (% dose per litre of plasma) at baseline and after 12 months in 198 Caucasian and African American women (age 25–45 years) with initial serum 25(OH)D concentrations ≤ 50 nmol/L. Participants received a vitamin D_3 supplementation dose of 10, 20, 40, 60 µg/day or placebo and were advised to take a calcium supplement (200 mg) to maintain a calcium intake of approximately 1,000 mg/day. Mean serum 25(OH)D increased from 33.5 nmol/L (all subjects) at baseline to 100 nmol/L in the group receiving the highest dose of vitamin D_3. No changes in calcium absorption were observed over time on any dose in either Caucasians or African Americans, and no significant relationship was observed between 12-month calcium absorption and baseline or final serum 25(OH)D. No threshold value of serum 25(OH)D for calcium absorption was found at baseline or in the longitudinal study. **The**

Panel notes that these two studies do not to identify a threshold for serum 25(OH)D concentration below which calcium absorption is impaired.

Conclusions on calcium absorption in adults

The Panel notes that all studies identified after the IOM report were conducted in women (mostly postmenopausal women), but otherwise quite heterogeneous with respect to study design (age profile of subjects, ethnicity, body weight, dose of vitamin D, calcium supplementation), which contribute to the mixed findings and limit a conclusion. Duration of RCTs ranged between six weeks and one year.

The Panel notes that the cross-sectional single-isotope study by Need et al. (2008), included in the review by the IOM, showed that calcium absorption was reduced at 25(OH)D concentrations around 10 nmol/L, below which the formation of $1,25(OH)_2D$ was compromised.

The Panel also notes that the two recent RCTs (Shapses et al., 2013; Aloia et al., 2014) and the one observational study (Shapses et al., 2012) using the dual isotope technique included subjects with relatively high baseline serum 25(OH)D concentrations (mean above 60 nmol/L). The Panel notes that these three studies showed no threshold value for serum 25(OH)D concentration in relation to fractional calcium absorption, in particular no threshold value in the serum 25(OH)D range between 40 and 130 nmol/L (Aloia et al., 2014) or that fractional calcium absorption was higher in the group (Shapses et al., 2012) with the lowest serum 25(OH)D concentration (mean 63 nmol/L). These results are supported by findings of two RCTs (Gallagher et al., 2012; Gallagher et al., 2014) using the single isotope technique and undertaken at lower baseline mean serum 25(OH)D concentrations (33.5 and 38 nmol/L). Results of studies are inconsistent on whether serum 25(OH)D concentration was a statistically significant predictor of calcium absorption (Gallagher et al., 2012; Aloia et al., 2014) or not.

Overall, based on these studies, the Panel considers that calcium absorption was shown to be compromised only in patients with vitamin D deficiency (i.e. serum 25(OH)D concentration $\leq$ 10 nmol/L) and that the recent studies provide no evidence of a threshold effect in relation to fractional calcium absorption in adults, for serum 25(OH)D concentrations ranging between 33.5 and 75 nmol/L (mean at baseline) or between 40 to 130 nmol/L (range of final concentrations).

5.1.2.1.7. Summary of conclusions on serum 25(OH)D concentration as indicator of musculoskeletal health in adults

The Panel notes that the evidence on a possible threshold value for serum 25(OH)D concentration with regard to adverse musculoskeletal health outcomes in adults shows a wide variability of results. Several factors contribute to this (Sections 5.1.1.1.1, 5.1.1.1.3, 5.1.1.1.4.) and also include the large variation in the results from different laboratories and assays used for measuring serum 25(OH)D concentrations (Section 2.4.1). Furthermore (as indicated in the previous sections), observational studies mostly used single measurements of 25(OH)D concentration, thus possible long-term changes in 25(OH)D concentration were not considered in the analyses of the relationship with health outcomes.

The Panel concludes that, regarding the relationship between serum 25(OH)D concentration and

- BMD/BMC in non-institutionalised adults, there is some evidence for a higher risk of increased BMD/BMC losses with serum 25(OH)D concentrations below 50 nmol/L,

- osteomalacia, there is limited evidence that the risk of vitamin D-deficiency osteomalacia is small with serum 25(OH)D concentrations at or above 50 nmol/L,

- fracture risk in non-institutionalised adults, the majority of studies indicate an increased risk for fractures associated with serum 25(OH)D concentrations of < 18 nmol/L to < 50 nmol/L,

- muscle strength/function and physical performance, the evidence is inconsistent, and no target value for 25(OH)D concentration with regard to muscle strength/function and physical performance can be derived,

- falls/falling, the evidence is mixed, but overall is suggestive of beneficial effects of vitamin D supplementation for reducing the risk of falls and falling in older adults over a range of serum 25(OH)D concentration (means of 23 to 82 nmol/L according to the systematic reviews considered). From the available evidence, no target value for 25(OH)D concentration with regard to the risk of falls or falling can be derived,

- calcium absorption, that a threshold below which fractional calcium absorption is compromised has been shown in patients with serum 25(OH)D concentrations around 10 nmol/L, and that there is no evidence of a threshold effect in relation to fractional calcium absorption in adults, for serum 25(OH)D concentrations above about 30 nmol/L.

5.1.2.2. Infants and children

5.1.2.2.1. Bone mineral density/content

IOM (2011) (Section 4 and Appendix B) noted the lack of data relating serum 25(OH)D concentration to bone accretion measures in infants, and that the evidence for an association between serum 25(OH)D concentration and BMC measures in infants was inconsistent. IOM (2011) noted that, in children above one year of age, serum 25(OH)D concentrations of 40–50 nmol/L 'would ideally coincide with bone health benefits such as positive effects on BMC and BMD' (Viljakainen et al., 2006b; Cranney et al., 2007; Chung et al., 2009). IOM (2011) also noted that the results of RCTs in children are inconsistent when compared to results of observational studies. Overall, the IOM considered that there was some evidence for a positive association between serum 25(OH)D concentration in children and baseline BMD or change in BMD.

Lamberg-Allardt et al. (2013) based their conclusions about the possible relationship between serum 25(OH)D concentration and BMC or BMD in infants and children on Cranney et al. (2007), and their conclusions were in agreement with those derived by IOM (2011).

Newberry et al. (2014) examined the effect of vitamin D supplementation on 25(OH)D concentration and BMC in infants or children (Molgaard et al., 2010; Holmlund-Suila et al., 2012; Khadilkar et al., 2012), and considered that there was no reason to change previous conclusions (Cranney et al., 2007; Chung et al., 2009).

In infants, SACN (2015) concluded that the evidence from four intervention studies (Kim M-J et al., 2010; Kumar et al., 2011a; Abrams et al., 2012; Holmlund-Suila et al., 2012), is inconsistent with regard to an effect of vitamin D supplementation on indices of bone health in infants. The SACN also noted some methodological limitations in one study (Kim MJ et al., 2010), and the specific population of another study (undernourished low birth–weight infants (Kumar et al., 2011b)). For bone health indices in children aged 1–3 years, the SACN identified a cross-sectional study (Hazell et al., 2015) on the relationship between plasma 25(OH)D and BMC/BMD, that is not a type of study usually considered by the Panel for this Section (Section 5.1.). For children aged above four years, SACN (2015) concluded that a systematic review and meta-analysis including six RCTs (Winzenberg et al., 2011)[26] (mean age: 10 to 13 years) reported a beneficial effect of vitamin D_3

[26] None of the included studies in this systematic review were published in 2010 or afterwards.

supplementation on total body BMC when baseline serum 25(OH)D concentration was < 35 nmol/L. However, the SACN noted that the 35 nmol/L cut-off value was arbitrarily selected based on the distribution of data (to have sufficient data for sub-group analyses). SACN (2015) also identified five trials on 'bone health indices', i.e. calcium absorption (Park et al., 2010), BMC/BMD (Molgaard et al., 2010; Ward et al., 2010; Khadilkar et al., 2012), marker of bone resorption (Ghazi et al., 2010) in children and adolescents. However, three of these studies used supplementation given monthly, bimonthly, or every third month (Ghazi et al., 2010; Ward et al., 2010; Khadilkar et al., 2012), which did not correspond to the inclusion criteria defined by the Panel for its literature search (Section 5.1.).

The Panel retrieved five intervention and prospective observational, reporting on BMD/BMC in infants/children in relation to 25(OH)D concentrations and that were published after the report by IOM (2011). In the following section, the *four intervention studies*, first in infants then in children, are described individually, followed by the *one prospective observational study* in children. The results are then summarized, and an *overall conclusion on* BMC/BMD in infants/children is provided

Trials with vitamin D supplementation

In a trial in 38 breastfed healthy infants (Hispanic and non-Hispanic) in the USA, who all received 10 µg/day vitamin D_3 supplementation for three months from one week after birth, Abrams et al. (2012) investigated changes in 25(OH)D concentration (cord blood then infant blood), BMC and BMD between baseline and follow-up. Mean 25(OH)D concentrations were 57.5 nmol/L (non-Hispanic) and 42 nmol/L (Hispanic) in cord blood, and were 94 nmol/L and 78 nmol/L, respectively, at age three months. There was no significant linear relationship between change in 25(OH)D and change in BMC. After adjustment for potential confounders, there was no significant relationship between cord 25(OH)D and BMC at three months. **The Panel** notes that, in this study of short duration (3 months), mean 25(OH)D concentration rose from about 42–58 nmol/L (cord blood) to 78-94 nmol/L at follow-up after daily vitamin D supplementation of all infants, but there was no relationship between cord 25(OH)D and BMC at three months.

In a double-blind randomised trial in 113 healthy term newborns (107 included in the analyses, among which 102 were breastfed infants) in Finland, Holmlund-Suila et al. (2012) investigated whether vitamin D_3 supplementation (10 µg/day or two other doses higher than the UL for infants, i.e. higher than 25 µg/day) from age two weeks to three months could ensure a serum 25(OH)D concentration of at least 80 nmol/L, without signs of excess. Samples of cord blood were collected at birth to measure baseline serum 25(OH)D, and tibia total and trabecular bone density or area, cortical bone density or area, and bone stress and strain index were assessed by pQCT (see Appendix A). Serum 25(OH)D measured at birth in cord blood did not differ among groups (mean : 52–54 nmol/L according to groups, median : 53 nmol/L in the whole population) and was 88 nmol/L (mean) at three months in the group receiving 10 µg/day, with a minimum value at three months of 46 nmol/L. After adjustment for potential confounders, there was no significant difference in bone parameters measured by pQCT between the three vitamin D-supplemented groups. **The Panel** notes that, in this study of short duration (2.5 months), mean serum 25(OH)D concentration rose from about 53 nmol/L (cord blood) to 88 nmol/L (in the group receiving the lowest dose) after vitamin D_3 supplementation in infants, but vitamin D_3 doses of 10 µg/day or higher did not result in differences in BMD.

In a double-blind randomised trial in Canada, 132 breast-fed infants aged ≤ 1 month received, for 11 months, vitamin D_3 supplementation at either 10, 20, 30 or 40 µg/day (two of these doses being higher than the UL for infants, i.e. higher than 25 µg/day) (Gallo et al., 2013). The primary outcome was to achieve a plasma 25(OH)D concentration of 75 nmol/L or greater in 97.5% of infants at three months. Whole body and regional BMC were included among the secondary outcomes and monitored at baseline, 3, 6, 9 and 12 months of age. Mean plasma 25(OH)D concentration was

59 nmol/L (95% CI, 55-63 nmol/L) across all groups at baseline and peaked in all groups at three months (at 78 and 102 nmol/L in the two groups with the lowest dose). While authors reported a dose-response relationship for vitamin D dosage and plasma 25(OH)D concentration, no such relationship was observed between vitamin D dosage and BMC (lumbar spine, femur, whole body) or BMD (lumbar spine) over time. **The Panel notes** that, in this study, mean plasma 25(OH)D concentration rose from 59 nmol/L to at least 78 nmol/L (at three months) after vitamin D_3 supplementation, but vitamin D_3 doses of 10 or 20 µg/day or higher did not result in differences in BMC/BMD over one year.

In a double-blind RCT (Molgaard et al., 2010), 225 Danish girls (221 completers) aged 11–12 years were randomised to vitamin D_3 (5 or 10 µg/day) or placebo over one year with the same study design as in (Viljakainen et al., 2006b) in Finnish girls (included in the review by IOM). However, Molgaard et al. (2010) recruited the subjects during all seasons, whereas Viljakainen et al. (2006b) recruited between October and March. Whole-body and lumbar spine BMC, bone area (BA) and BMD were measured by DXA at baseline and after 12 months. Mean serum 25(OH)D (about 42-44 nmol/L) or bone measures did not differ between groups at baseline. Adjusting for baseline values, the 12-month mean change in serum 25(OH)D concentration was significantly different between groups ($p < 0.0001$), being 39.7 nmol/L (-3.1 nmol/L from baseline) in the placebo group and 52.9 and 57.9 nmol/L (+ 11 and + 13.3 nmol/L from baseline) in the 5 µg and 10 µg groups, respectively. The intervention had no effect on total body and lumbar spine BMC, BMD or BA in the whole population compared with placebo, except for the lumbar spine BA ($p = 0.039$, with the lowest increase in the group supplemented with 10 µg/day). **The Panel notes** that, in this RCT in prepubertal and pubertal girls, raising mean serum 25(OH)D concentration from 42–44 nmol/L to 53–58 nmol/L by two daily vitamin D_3 supplementation (compared with placebo) did not result in changes in BMD or BMC after one-year.

Prospective observational study

In a UK prospective cohort study in Caucasian children (n = 2 247 in fully adjusted analyses), Sayers et al. (2012) investigated the relationship between plasma 25(OH)D_2 or 25(OH)D_3 concentrations and a number of pQCT measures (cortical BA, cortical BMC, cortical BMD, periosteal circumference, endosteal circumference and cortical thickness) (Appendix A) of the mid-tibia at age 15.5 years. Plasma 25(OH)D concentrations from samples collected at the age of 9.9 years were considered in the analysis, or at the age of 11.8 or 7.6 years if measurement at age 9.9 years was not available. Mean baseline plasma 25(OH)D_3 concentration was about 57-60 nmol/L in boys and girls, and mean 25(OH)D_2 concentration was about 4.5 nmol/L in both genders. Plasma 25(OH)D_3 concentration at baseline was significantly associated with to endosteal adjusted for periosteal circumference (negatively); cortical BMC, cortical BA or cortical thickness (positively), after adjustment for potential confounders. **The Panel notes** that in this study in children with a mean baseline plasma 25(OH)D_3 concentration of about 57–60 nmol/L, plasma 25(OH)D_3 concentration was significantly associated with several bone measures.

Conclusions on BMC/BMD in infants/children

In *infants*, the Panel found three recent trials on BMD or BMC in (mostly) breastfed infants, two of short duration (three months of less) (Abrams et al., 2012; Holmlund-Suila et al., 2012) and one of 11 months (Gallo et al., 2013). One trial did not show any relationship between baseline or change in mean 25(OH)D concentration (from 42–58 nmol/L (cord) up to 78–94 nmol/L) after vitamin D supplementation and BMC at three months (Abrams et al., 2012). After different daily doses of vitamin D supplementations, the two others did not show that increasing mean serum 25(OH)D concentrations from about 53 nmol/L (cord) (Holmlund-Suila et al., 2012) or 59 nmol/L (≤ 1 month) (Gallo et al., 2013), up to means at three months of at least 88 nmol/L or at least 78 nmol/L, respectively, resulted in differences on BMD/BMC (at age three (Holmlund-Suila et al., 2012) or twelve (Gallo et al., 2013) months).

For *children*, the only RCT, undertaken in prepubertal and pubertal girls, showed that raising mean serum 25(OH)D concentration from 42–44 nmol/L to 53–58 nmol/L by two daily doses of vitamin D_3 supplementation (compared with placebo) did not result in changes in BMD or BMC after one-year (Molgaard et al., 2010). In one prospective cohort study in children with a mean baseline plasma 25(OH)D_3 concentration of about 57–60 nmol/L, plasma 25(OH)D_3 concentration was significantly associated with several bone measures (Sayers et al., 2012).

The Panel took into account the conclusions by IOM on the relationship between serum 25(OH)D concentrations and BMC/BMD in infants (inconsistent results) and children (evidence for a positive association), and the studies published thereafter. **Overall, the Panel considers** that there is some evidence that, in infants and children, increasing mean serum 25(OH)D from about 40–60 nmol/L to higher values is not associated with further benefit on BMC/BMD.

5.1.2.2.2. Rickets

IOM (2011) (Section 4 and Appendix B) considered, that *in the presence of an adequate calcium intake*, there was evidence for an association between low mean serum 25(OH)D concentration (< 30 nmol/L) and confirmed rickets (Section 2.2.2.1.) and that the risk of rickets was 'minimal when serum 25(OH)D levels range between 30 and 50 nmol/L'.

Based on Cranney et al. (2007), Lamberg-Allardt et al. (2013) concluded that there was an increased risk of rickets below a serum 25(OH)D concentration of 27.5 nmol/L, i.e. about 30 nmol/L. No new data on rickets were identified by Newberry et al. (2014). SACN (2015) concluded that the evidence from a total of 40 studies (including case reports) , on vitamin D and rickets is mainly observational and therefore subject to confounding. The SACN notes that most studies did not report on calcium intake, thus it was unclear if rickets was caused by vitamin D deficiency or by low calcium intake or both, and that most studies did not provide information on the time of year in which the blood sample was drawn. The SACN reported that serum 25(OH)D concentration in case reports ranged from < 2.5 to < 50 nmol/L and that mean/median concentrations ranged between 5 and 50 nmol/L in other study types in patients. Individual and mean serum 25(OH)D concentrations were < 25 nmol/L in the majority of studies examined.

The Panel did not find any relevant primary study on serum 25(OH)D and the risk of rickets in infants and children, providing information on their calcium intake and published after the IOM report.

The Panel takes into account the conclusions by IOM (2011) and Lamberg-Allardt et al. (2013) on evidence of overt rickets at mean serum 25(OH)D concentrations below 30 nmol/L with adequate calcium intake. Based on conclusions by IOM that the risk of rickets was minimal when serum 25(OH)D concentration ranges between 30 and 50 nmol/L, **the Panel concludes** that there is no risk of vitamin D-deficiency rickets with serum 25(OH)D concentrations **at or above 50 nmol/L** and adequate calcium intake.

5.1.2.2.3. Calcium absorption

IOM (2011) reviewed together data on calcium absorption in adults or children (Sections 4 and 5.1.2.1.6., Appendix B). The IOM concluded that, in life stages of bone accretion, maximal calcium absorption is associated with serum 25(OH)D concentrations of at least 30 nmol/L, and closer to 40 to 50 nmol/L, and that fractional calcium absorption does not appear to increase with serum 25(OH)D concentration above 50 nmol/L. The Panel notes that the IOM included the study by Abrams et al. (2009), which pooled studies in 251 children (about 5–17 years) using the dual isotope technique. This study found that, when serum 25(OH)D concentration was studied as a categorical variable in the whole population, fractional calcium absorption adjusted (in particular)

for calcium intake was slightly but significantly higher at serum 25(OH)D concentration of 28-50 nmol/L (0.344 ± 0.019), compared with concentrations of 50-80 nmol/L (0.280 ± 0.014, $p < 0.001$) or greater than 80 nmol/L (0.297 ± 0.015, $p < 0.007$). Calcium absorption was not considered 'as such' by Lamberg-Allardt et al. (2013), Newberry et al. (2014) or SACN (2015). However SACN (2015) considered the trial by Park et al. (2010) on fractional calcium absorption (described below).

The Panel identified one additional RCT (Abrams et al., 2013) using the dual-stable isotope technique for measuring fractional calcium absorption. As for studies on calcium absorption in adults (Section 5.1.1.1.6.), the Panel also considered two studies (Park et al., 2010; Lewis et al., 2013) using the single isotope technique (considered as supportive evidence by the Panel and described below).

With regard to results obtained with the dual isotope technique, in an eight-week RCT in 63 prepubertal children aged 4–8.9 years consuming 600 to 1,200 mg/day calcium at baseline and who received 25 µg/day vitamin D_3 or a placebo (Abrams et al., 2013), mean 25(OH)D concentration was about 70 nmol/L in both groups at baseline and was significantly lower (mean ± SD: 75 ± 12 nmol/L) in the placebo than in the supplemented group (90 ± 6 nmol/L) ($p = 0.01$) at the end of the study period. No significant difference in fractional calcium absorption was measured at baseline and at the end of the study between the placebo group and the vitamin D_3 supplemented group. **The Panel notes** that, in this study, increasing mean serum 25(OH)D from 70 to 90 nmol/L by vitamin D supplementation (compared with placebo) did not result in any difference in fractional calcium absorption.

With regard to results obtained with the single-isotope technique, Park et al. (2010) used a two-period metabolic balance study to investigate the effect of vitamin D supplementation on calcium absorption and retention in 11 adolescent girls aged 12–14 years with a mean entry serum 25(OH)D concentration of 35.1 nmol/L. Subjects consumed a controlled intake (providing 5 mg vitamin D and 1,117 mg calcium/day) for two three-week metabolic balance periods separated by a one-week washout period. After the first metabolic balance period, participants received 25 mg/day vitamin D_3 supplementation for four weeks. Fractional calcium absorption was measured in each metabolic balance period using a stable calcium isotope method. All urine and faecal samples were collected and analyzed to measure net calcium absorption and calcium retention. Daily supplementation with 25 mg vitamin D resulted in a mean increase in serum 25(OH)D of 13.3 nmol/L ($p < 0.01$) but a decrease in fractional calcium absorption of 8.3% ($p < 0.05$) and no significant change in fasting serum $1,25(OH)_2D$, PTH, net calcium absorption, or calcium skeletal retention. **The Panel notes** that, in this study in pubertal girls, increasing mean serum 25(OH)D from 35.1 nmol/L to 48.4 nmol/L did not improve fractional or net calcium absorption.

In a 12-week double-blind RCT in children aged 9–13 years (165 African American and 158 Caucasian) with a mean baseline calcium intake of about 900 mg/day, Lewis et al. (2013) evaluated the effects of daily vitamin D_3 supplementation (10 µg, 25 µg, 50 µg, 100 µg) or placebo on 25(OH)D concentration and other parameters including fractional calcium absorption. Compared with a mean baseline 25(OH)D concentration of 70 nmol/L in the whole population, the mean change in 25(OH)D was - 10 nmol/L for the placebo group, and ranged from + 5.5 nmol/L to + 76.1 nmol/L in the supplemented groups. In the whole population, 25(OH)D concentration at baseline or after 12 weeks was not related to changes in fractional calcium absorption, even after adjustment for potential confounders. There was no effect of vitamin D_3 supplementation on change in fractional calcium absorption. **The Panel notes that,** in this study, 25(OH)D concentration at baseline (mean: 70 nmol/L) or after 12 weeks of vitamin D supplementations compared with placebo was not related to changes in fractional calcium absorption.

Conclusions on calcium absorption in children

The Panel notes that few data are available on the relationship between serum 25(OH)D concentration and fractional calcium absorption in children.

The Panel notes that the dual-isotope study by Abrams et al. (2009), included in the review by the IOM, showed that fractional calcium absorption was slightly but significantly higher at serum 25(OH)D concentration of 28–50 nmol/L (0.344 ± 0.019), compared with concentrations of 50-80 nmol/L (0.280 ± 0.014, $p < 0.001$) or greater than 80 nmol/L (0.297 ± 0.015, $p < 0.007$), among children of 5 to 17 years of age. The Panel also took into account a metabolic balance study in adolescent girls (Park et al., 2010) showing that increasing mean serum 25(OH)D from 35.1 nmol/L to 48.4 nmol/L did not improve fractional or net calcium absorption. In addition, the Panel notes that the two recent RCTs using the dual isotope technique (Abrams et al., 2013) or the single isotope technique (Lewis et al., 2013) in children with relatively high baseline serum 25(OH)D concentrations (mean about 70 nmol/L) did not find any relationship between fractional calcium absorption and serum 25(OH)D concentration (or any threshold value for this concentration).

Overall, based on these studies, the Panel considers that there is no relationship between fractional calcium absorption in children and serum 25(OH)D concentration above about 30-50 nmol/L.

5.1.2.2.4. Summary of conclusions on serum 25(OH)D concentration as indicator of musculoskeletal health in infants and children

The Panel notes the paucity of data on serum 25(OH)D concentrations and musculoskeletal health outcomes in infants and children.

In spite of the large variation in the results from different laboratories and assays used for measuring serum 25(OH)D concentrations (Section 2.4.1), the Panel nevertheless concludes that, regarding the relationship between serum 25(OH)D concentration and

- BMD/BMC in infants and children, there is some evidence that, in infants and children, increasing mean serum 25(OH)D from about 40–60 nmol/L to higher values is not associated with further benefit on BMC/BMD,

- rickets, there is no risk of vitamin D-deficiency rickets with serum 25(OH)D concentrations at or above 50 nmol/L and adequate calcium intake,

- calcium absorption, there is no relationship between fractional calcium absorption in children and serum 25(OH)D concentration above about 30-50 nmol/L.

The Panel considers that the evidence on associations between serum 25(OH)D and musculoskeletal health outcomes is not adequate to set a different target value for serum 25(OH)D concentration in children compared to adults.

5.1.3. Serum 25(OH)D concentration and health outcomes in pregnancy

IOM (2011) (Section 4 and Appendix B) considered the following outcomes for pregnancy: calcium absorption, maternal/fetal/neonatal/childhood bone health and related outcomes (e.g. PTH), neonatal rickets, and maternal blood 25(OH)D. Separately, the IOM also considered pre-eclampsia (i.e. hypertension with proteinuria) and pregnancy-induced hypertension (i.e. transient hypertension without proteinuria). IOM (2011) concluded that calcium absorption, maternal bone health, neonatal rickets, risk of pre-eclampsia or pregnancy-induced hypertension, or non-skeletal (maternal or

infant) outcomes could not be used to set DRVs for vitamin D for pregnant women. IOM concluded that fetal and childhood bone-related health outcomes were informative for the development of reference values for vitamin D in pregnancy, which in the end did not differ from that for non-pregnant women.

Newberry et al. (2014) identified one article in relation to pre-eclampsia, that reported on two combined RCTs assessing the effect of supplemental vitamin D (Wagner et al., 2013b). They also refer to five nested case-control studies (Baker et al., 2010; Powe et al., 2010; Shand et al., 2010; Woodham et al., 2011; Wei et al., 2012) and two prospective cohort studies (Scholl et al., 2013; Wei et al., 2013). Newberry et al. (2014) noted that some recent studies suggest a possible relationship between vitamin D supplementation or status and the risk of preeclampsia. Newberry et al. (2014) identified two cohort studies published after the report by IOM, that assessed the association between maternal serum 25(OH)D concentrations and the risk of giving birth to a small-for-gestational-age (SGA) infant (Bodnar et al., 2010; Burris et al., 2012) ((Bodnar et al., 2010) being already included in the IOM report). Newberry et al. (2014) also identified one nested case-control study and one prospective cohort study that assessed the association with preterm birth (Baker et al., 2011; Bodnar et al., 2013), of which one study was conducted in women with twin pregnancy (Bodnar et al., 2013).

SACN (2015) identified one cohort study (Haliloglu et al., 2011) on marker of bone turnover in pregnancy and post partum and five cohort studies (Prentice et al., 2009; Mahon et al., 2010; Viljakainen et al., 2010; Dror et al., 2012; Young et al., 2012) (some of them included in the IOM report, and some of them using pre-determined cut-offs for serum 25(OH)D)). The SACN reported that four of the cohort studies showed a positive association between maternal serum 25(OH)D concentration and various 'indices of bone health' in the fetus (Mahon et al., 2010; Young et al., 2012) or newborn (tibia BMC and cross-sectional area CSA (Viljakainen et al., 2010), cord serum bone specific ALP and cord serum 25(OH)D (Dror et al., 2012)). SACN (2015) also considered maternal serum 25(OH)D concentration in relation to non-skeletal outcomes in the mother as well as in the newborn. SACN (2015) also considered evidence from a systematic review (Harvey et al., 2014), which reported that the association between maternal serum 25(OH)D concentration during pregnancy and pre-eclampsia and gestational diabetes is inconsistent.

The Panel undertook a literature search and also reviewed recent primary studies identified in two systematic reviews of intervention and observational studies (Harvey et al., 2014; Newberry et al., 2014). As for other adults and children, markers of bone formation and turnover (e.g. (Haliloglu et al., 2011; Dror et al., 2012)) were not an outcome considered by the Panel in view of setting DRVs for vitamin D ((Section 5.1.1.).

Regarding the review health outcomes in pregnancy, **with the aim of setting DRVs for vitamin D**:

- **The Panel considered** available primary studies (RCTs and prospective observational studies) on serum 25(OH)D during pregnancy and **maternal outcomes** (bone health, for which no new data were found, pre-eclampsia or pregnancy induced hypertension). The Panel also considered the relationship between serum 25(OH)D during pregnancy and the following **outcomes in the newborn or child (but not in the fetus):** bone health at birth, gestational length, anthropometry at birth in relation to the risk of SGA, risk of preterm birth, bone health/anthropometry/body composition in the first year of life.

- In addition, the **Panel did not consider** studies providing risk estimates in specific populations like women with type 1 diabetes (Azar et al., 2011), patients already with pre-eclampsia or women all recruited for being at high risk of pre-eclampsia (Shand et al., 2010; Robinson et al., 2011), or studies with supplementation of other nutrients besides vitamin D but without measurement of 25(OH)D concentration (Watson and McDonald, 2010). In addition, the Panel did not consider data on adolescent or twin pregnancies (Bodnar et al.,

2013). The Panel also did not consider further investigations of studies mentioned below (Woodham et al., 2011; Wei et al., 2013), as they investigated the combined association of angiogenesis and endothelial dysfunction indicators, in addition to serum 25(OH)D concentration, with the risk of preeclampsia.

The Panel identified a total of 12 references on maternal 25(OH)D concentration and: *risk of pre-eclampsia*, *risk of being born SGA*, *risk of preterm birth*, and *bone health of the offspring*.

Some studies identified considered several of these outcomes. In the following section, *for each of these outcomes* (Sections 5.1.2.1. to 5.1.2.4.), the studies are described individually below; the results are then summarized, and an conclusion on maternal 25(OH)D concentration and the considered outcome is proposed. Finally, an *overall conclusion* for health outcomes in pregnancy is provided (Section 5.1.2.5.).

5.1.3.1. Risk of pre-eclampsia

The Panel identified only two intervention studies with vitamin D during pregnancy and several outcomes including birth weight and the risk of pre-term birth or pre-eclampsia, reported in one reference (Wagner et al., 2013b). The other six pertinent references on the risk of pre-eclampsia identified were observational studies and are described afterwards.

RCTs with vitamin D supplementation

Wagner et al. (2013b) combined datasets (total n = 504, age ≥ 16 years) from two double-blind RCTs (Hollis et al., 2011; Wagner et al., 2013a) on healthy women at 12 to 16 weeks of pregnancy and followed until delivery. All subjects received a prenatal 10 µg/day vitamin D_3 supplement, and were randomised to receive either a placebo, or daily doses of vitamin D_3 supplements (to reach a total intake of 50 or 100 µg/day). Serum 25(OH)D concentrations were not statistically different between groups (means between 57 and 65 nmol/L) at baseline (during pregnancy), but were higher in the supplemented groups compared to control in maternal blood within six weeks of delivery or in neonatal/cord blood, after adjustments for potential confounders. Four main Comorbidities Of Pregnancy (COPs), including pre-eclampsia and related hypertensive disorders as well as preterm birth without pre-eclampsia, were investigated as secondary outcomes. The study showed that the OR of any COP per 25 nmol/L increment of final maternal 25(OH)D concentration did not reach statistical significance, (but the risk was significantly reduced when all COPs were considered together). Neonatal birth weight did not significantly differ between supplemented groups and controls. **The Panel notes** that there was no effect of daily supplementation with vitamin D_3 during pregnancy on neonatal birth weight, and risk of pre-eclampsia or preterm birth in this population with mean serum 25(OH)D concentrations of 57–65 nmol/L at baseline.

Prospective observational studies

In the following observational studies, pre-eclampsia was defined at the occurrence of gestational hypertension in previously normotensive women accompanied by new-onset proteinuria after 20 weeks of gestation. Definition of pre-eclampsia based on values of systolic and/or diastolic blood pressure and proteinuria, although close, differed between studies, and severe pre-eclampsia was defined based on higher values of systolic BP/ diastolic blood pressure or proteinuria.

In a nested case-control study in the USA, Powe et al. (2010) assessed the association between first trimester total serum 25(OH)D concentrations and development of pre-eclampsia in 39 cases (with a significantly higher first trimester systolic and diastolic blood pressure), and 131 normotensive control women (who remained normotensive in pregnancy, did not have gestational diabetes mellitus or did not give birth to SGA infants). Baseline serum 25(OH)D concentrations did not differ significantly between cases and controls (mean about 68 and 72 nmol/L, respectively,

measured at (mean ± SD) 11.2 ± 3.6 versus 11.6 ± 3.0 weeks of gestation) and were not associated with baseline systolic or diastolic blood pressure. No association was found between first trimester serum 25(OH)D concentration (per 25 nmol/L increase, across quartiles, or for those < or > 37.5 nmol/L) and risk of subsequent pre-eclampsia, after full adjustments for potential confounders. **The Panel notes** that this study did not report an association between serum 25(OH)D concentration during the first trimester of pregnancy and incidence of pre-eclampsia.

One nested case-control study by Baker et al. (2010) was conducted in the USA in a population selected from a cohort of 3,992 healthy women, who had previously given blood in the framework of a routine prenatal care. The study analysed maternal 25(OH)D status during mid-gestation (15-20 weeks of gestation) and risk of development of severe pre-eclampsia. From the cohort, a case group of 51 women was identified who developed severe pre-eclampsia (median age 28 years), out of which 41 women were included in the analysis. The control group was composed of 198 randomly-selected ethnicity-matched healthy women delivering at term. Median serum 25(OH)D concentration in the case group was 75 nmol/L, which was significantly lower than that in the control group, i.e. 98 nmol/L. After adjustment for potential confounders, the risk of severe pre-eclampsia in women with mid-gestation 25(OH)D concentration of less than 50 nmol/L (n = 19 controls and 11 women with severe pre-eclampsia) was five-fold higher (OR: 5.41; 95% CI: 2.02–14.52) than in women with mid-gestation 25(OH)D of at least 75 nmol/L (n = 138 controls and 22 women with severe pre-eclampsia). There was no significant difference in risk in women with 25(OH)D between 50 and 74.9 nmol/L (n = 41 controls, and 10 with severe pre-eclampsia) compared with 25(OH)D of at least 75 nmol/L. **The Panel notes** that this study found that the risk for severe pre-eclampsia was higher in women with a 25(OH)D concentration at 15–20 weeks of gestation less than 50 nmol/L in comparison to those with concentrations higher than 75 nmol/L.

In a case-control study in the USA, Robinson et al. (2010) investigated maternal plasma 25(OH)D concentration in 50 women with diagnosed early-onset severe pre-eclampsia (EOSP, diagnosed before 34 weeks of gestation) compared to 100 ethnicity- and gestational age-matched healthy controls followed throughout their normal singleton pregnancy. Plasma 25(OH)D concentration (median (interquartile range IQR)), was obtained in the cases at time of diagnosis (45 (32.5-77.5) nmol/L) and was significantly lower than in controls (80 (50–110) nmol/L; $p < 0.001$), both at a mean gestational age of 29 weeks (28–31 weeks in cases, 26-31 weeks in controls). Birth weight and gestational age at delivery were significantly lower in cases than in controls, whilst mean arterial pressure at sample collection and incidence of intrauterine growth restriction (i.e. less than 10th percentile birth weight for gestational age) were significantly higher. After adjustment for potential confounders, there was a significant association between a 25 nmol/L increase in maternal plasma 25(OH)D and a reduced risk of EOSP (OR: 0.37; 95% CI: 0.22–0.62, $p < 0.001$). Women with plasma 25(OH)D concentration ≤ 49 nmol/L (lowest quartile) had a 3.6-fold increased risk of EOSP compared to women with higher concentrations (OR: 3.60; 95% CI: 1.71–7.58, $p < 0.001$). **The Panel notes** that this study indicates that the risk for early-onset severe pre-eclampsia was 3.6-time higher in women with a plasma 25(OH)D concentration at about 34 weeks of gestation less than 50 nmol/L in comparison with women with higher plasma 25(OH)D concentrations.

In a Spanish prospective cohort study in unsupplemented women followed from pregnancy to delivery (n = 466 at delivery), Fernandez-Alonso et al. (2012) investigated the relationship between first-trimester serum 25(OH)D concentration and obstetric and neonatal pregnancy outcomes. These included pre-eclampsia, gestational hypertension, preterm birth (i.e. birth at 21–37 weeks of pregnancy), and number of SGA infants (i.e. with birth weights below the 10th percentile for gestational age). Serum 25(OH)D concentration at 11–14 weeks of pregnancy was below 50 nmol/L, between 50 and 74 nmol/L or at least 75 nmol/L for, respectively, 109, 191 and 166 women. No significant non-parametric correlations were found between the first-trimester

25(OH)D levels and several numeric obstetric or neonatal outcome variables. **The Panel notes** that this study only assessed correlations between 25(OH)D levels and obstetric or neonatal outcomes.

One prospective cohort study (post-hoc analyses) (Wei et al., 2012) was carried out on a group of 697 Canadian women that had previously participated during pregnancy in a multicentre trial of vitamin C and E supplementation and prevention of pre-eclampsia. The study investigated the association between maternal 25(OH)D concentrations and risk of pre-eclampsia. The subjects with at least one of four risk factors for pre-eclampsia identified by the authors were stratified in the "high-risk" group (n = 229), while nulliparous women without risk factors for pre-eclampsia were in the "low-risk" group (n = 468). Plasma 25(OH)D concentration was measured in maternal blood samples collected during the trial at visit 1 (entry, 12–18 weeks of gestation) and visit 2 (24-26 weeks of gestation). The difference between maternal mean 25(OH)D concentrations in preeclamptic (n = 32) and non-preeclamptic (n = 665) women was not statistically significant at visit 1 (about 51-56.0 nmol/L), but significant at visit 2 (48.9 ± 16.8 nmol/L versus 57.0 ± 19.1 nmol/L, p = 0.03). After adjustments for potential confounders, the risk of pre-eclampsia associated with maternal 25(OH)D < 50 nmol/L at 24–26 weeks of gestation (n = 236, including 19 preeclamptic) was 3.2-fold higher (OR: 3.24; 95% CI: 1.37–7.69) compared with maternal 25(OH)D ≥ 50 nmol/L (n = 368, 9 preeclamptic). This relationship was not observed for maternal 25(OH)D < 50 nmol/L (n = 272, 15 preeclamptic) or ≥ 50 nmol/L (n = 425, 17 preeclamptic) earlier in pregnancy, i.e. at 12–18 weeks of gestation. **The Panel notes** that according to these study findings, the risk of pre-eclampsia associated with maternal 25(OH)D concentration < 50 nmol/L at 24–26 weeks of gestation (but not at 12–18 weeks) was significantly higher compared with maternal 25(OH)D ≥ 50 nmol/L.

In a prospective cohort study on 1,141 US healthy pregnant women (mainly Hispanic and African American), Scholl et al. (2013) analysed the association of serum 25(OH)D concentration < 50 nmol/L (with or without PTH > 6.82 pmol/L) and the risk of pre-eclampsia. Maternal serum 25(OH)D concentration was measured at (mean ± SD) 13.7 ± 5.7 weeks of gestation, as $25(OH)D_3$ and $25(OH)D_2$, but mean baseline value was not reported. About 6% of women developed pre-eclampsia. After adjustment for potential confounders, and compared with women with 25(OH)D concentration of at least 50 nmol/L (n = 750), the risk of pre-eclampsia was significantly two-fold higher in pregnant women with concentrations lower than 30 nmol/L or between 30 and 39 nmol/L (n = 121 and 116, respectively, e.g. adjusted OR for 25(OH)D < 30 nmol/L: 2.13; 95% CI: 1.07–4.26, p for trend = 0.027) (but the risk was not significantly reduced in the 154 women with 25(OH)D of 40-50 nmol/L). Women with secondary hyperparathyroidism (n = 72, PTH > 6.82 pmol/L and serum 25(OH)D < 50 nmol/L) had a 2.8-fold increase in risk (95% CI: 1.28–6.41). **The Panel notes** that, according to this cohort study in mainly Hispanic and African American women, the risk of pre-eclampsia was about two-fold higher when the 25(OH)D concentration of the mother at 13.7 ± 5.7 weeks of gestation was < 40 nmol/L compared to those with a concentration ≥ 50 nmol/L.

Conclusions on risk of pre-eclampsia

The Panel notes that an increase in serum 25(OH)D concentration from a mean baseline of 57-65 nmol/L (after vitamin D supplementation in the second trimester of pregnancy compared with placebo) did not result in a change in the risk of pre-eclampsia (Wagner et al., 2013b). Out of six observational studies, two (Powe et al., 2010; Fernandez-Alonso et al., 2012) found no association between serum 25(OH)D during pregnancy (at time points of about 11-14 weeks of gestation), and risk of pre-eclampsia. In these two studies, investigated (pre-defined) cut-offs for 25(OH)D were < 37.5 and 50 nmol/L (versus > 37.5 or > 75 nmol/L). In contrast, four observational studies (Baker et al., 2010; Robinson et al., 2010; Wei et al., 2012; Scholl et al., 2013) found a significant association between low maternal serum 25(OH)D concentration (measured between about 13 to 31 weeks of gestation) and risk of pre-eclampsia or severe pre-eclampsia. In these studies, the investigated cut-offs, often pre-defined, were < 30 nmol/L, of 30-39 nmol/L or < 50 nmol/L,

compared most often with > 50 nmol/L (or ≥ 75 nmol/L). **Overall, the Panel considers** that the evidence of an association between maternal serum 25(OH)D concentration and risk of pre-eclampsia is inconsistent, although there is some evidence suggestive of an increase in the risk of pre-eclampsia at 25(OH)D concentrations below about 50 nmol/L.

5.1.3.2. Risk of being born small-for-gestational-age

With regard to the risk of being born SGA, the Panel considered four observational studies, including the study by Fernandez-Alonso et al. (2012) mentioned above.

Prospective observational studies

In a prospective population-based cohort study on 203 healthy Danish Caucasian women (Moller et al., 2012), the association between pre-conception 25(OH)D concentration and several outcomes was investigated. Outcomes included incidence of miscarriage and birth outcomes (birth weight and length, head circumference, number of SGA infants), and 153 women with immediate pregnancy plans were compared to 75 women who had no pregnancy plans for the next 21 months as age-matched controls (50 completers). Plasma 25(OH)D concentration was measured in both groups on four occasions (at baseline, and once at each of the follow-up visits every trimester). Median (IQR) baseline plasma 25(OH)D concentration (70 (56-92) nmol/L) was significantly ($p < 0.001$) higher in the control group compared to women with pregnancy plans (59 (46-71) nmol/L). Baseline mean plasma 25(OH)D concentrations did not differ between those who experienced miscarriage (n = 8) and those who did not. Plasma 25(OH)D concentration (at baseline, at each visit, or on average during pregnancy) was not associated with gestational length, birth weight, birth length, head circumference, incidence of SGA infants, even after adjustments for potential confounders. **The Panel** notes that this study, in a population with baseline median plasma 25(OH)D concentration of about 50-70 nmol/L, did not find an association between maternal 25(OH)D concentration during pregnancy and anthropometric outcomes in the newborn or SGA incidence.

In a prospective cohort study of pregnant women in the US, Burris et al. (2012) assessed the association between second trimester maternal plasma 25(OH)D concentration (947 Caucasians, 186 African Americans) or cord plasma 25(OH)D concentration (606 Caucasians, 128 African Americans) and the risk of SGA. Women were included at less than 22 weeks of singleton pregnancies. Mean ± SD maternal and cord 25(OH)D concentrations were 60 ± 21 (at 26–28 weeks of gestation) and 47 ± 19 nmol/L, respectively, and there were 53 SGA infants. After adjustments for potential confounders, maternal or cord plasma 25(OH)D < 25 nmol/L was associated with a significantly increased risk of SGA, compared with plasma 25(OH)D of 25 nmol/L or greater. Indeed, the adjusted OR of SGA was 3.17 (95% CI: 1.16–8.63) for maternal plasma < 25 nmol/L (7 SGA infants from mothers in this category), and 4.64 (95% CI: 1.61–13.36) for cord plasma < 25 nmol/L (9 SGA infants in this category). **The Panel** notes that this study in second trimester pregnant women showed that maternal or cord plasma/serum 25(OH)D concentrations below 25 nmol/L (versus at least 25 nmol/L) were associated with increased risk of SGA.

In a U.S prospective cohort study, Gernand et al. (2013) studied 2,146 pairs of singleton term newborns and mothers (52 % Caucasian, with no pre-existing diabetes or hypertension) who had participated in a large multicentre observational study (63% study sites at latitude ≥ 41° North). The aim of the study was to investigate the association between maternal 25(OH)D concentration and several outcomes, including the risk of SGA. Maternal serum 25(OH)D concentration was measured at 26 weeks of gestation or less, and every eight weeks afterwards (mean baseline: 51.3 ± 28.0 nmol/L). There were 395 SGA infants. After adjustments for potential confounders, the risk of SGA was half in infants whose mothers had first trimester 25(OH)D of ≥ 37.5 nmol/L, compared to < 37.5 nmol/L (OR:0.50; 95% CI: 0.27–0.91) (11.8 and 23.8 % of SGA infants from mothers in each category). This association was not observed in the second trimester. **The Panel**

notes that this study showed that maternal serum 25(OH)D concentrations above 37.5 nmol/L in the first trimester of pregnancy, but not the second trimester, were associated with half the risk of SGA compared with serum concentrations below 37.5 nmol/L.

Conclusions on risk of being born SGA

The Panel notes that, in contrast to Fernandez-Alonso et al. (2012) and Moller et al. (2012) (which measured frequency), two larger observational studies (Burris et al., 2012; Gernand et al., 2013) using pre-defined 25(OH)D cut-off values found an association of maternal 25(OH)D < 25 nmol/L (at 26–28 weeks of gestation) or < 37.5 nmol/L (in the first trimester, but not the second) with an increased risk of SGA (versus higher values). The Panel concludes that the evidence of an association between maternal serum 25(OH)D concentration and risk of being born SGA is inconsistent, although there is some evidence suggestive of an increase in the risk at 25(OH)D concentrations below about 25–37.5 nmol/L.

5.1.3.3. Risk of preterm birth

With regard to the risk of preterm birth, in addition to the two intervention studies reported in one reference (Wagner et al., 2013b) already described above (Section 5.1.2.1.), the Panel identified one nested case-control study.

Baker et al. (2011) assessed the relationship between maternal 25(OH)D concentration during pregnancy and the risk of preterm birth in a U.S nested case-control study of 4,225 women with singleton pregnancies, from whom blood had been collected at 11–14 weeks of gestation for the screening of trisomy 21. Preterm birth was defined as spontaneous delivery between 23 and 35 weeks of gestation. 40 women with pre-term birth were compared to ethnicity-matched randomly selected healthy controls who delivered at term (n = 120) and gave blood at a similar gestational age. Median (IQR) serum 25(OH)D concentration for the whole study group was 89 (73-106) nmol/L. After adjustment for potential confounders, there was no association between maternal serum 25(OH)D concentration (< 50 nmol/L or 50–74.9 nmol/L, compared with ≥ 75 nmol/L) and the risk of preterm birth. **The Panel notes** that this study found no association between 25(OH)D concentration during pregnancy and the risk for pre-term birth in this population with high baseline median 25(OH)D value (about 90 nmol/L).

5.1.3.4. Bone health of the offspring

With regard to bone health of the offspring, the Panel considered one observational study.

Viljakainen et al. (2011) evaluated in a Finnish prospective cohort study, whether there was a catch-up in tibia BMC or CSA in children (n = 87) at 14 months, from a group of 125 children previously assessed at birth (Viljakainen et al., 2010). These infants had been categorised according to maternal vitamin D status during pregnancy (defined as the mean of the first-trimester and of the two-day post-partum serum 25(OH)D concentrations below or above the median of 42.6 nmol/L). BMD, BMC and CSA of the left tibia were measured in the newborns and at 14 months by pQCT (Appendix A). Complete baseline and follow-up data were available for 29 and 26 children whose mothers had, respectively, lower or higher vitamin D status during pregnancy. Whereas tibia BMC at birth was significantly higher in children whose mothers had a high (i.e. above median) vitamin D status during pregnancy (Viljakainen et al., 2010), the mean total BMC gain over 14 months was significantly higher in the children whose mothers had a low vitamin D status (0.062 g/cm^2, p = 0.032) resulting in similar BMC in both groups of children at 14 months (Viljakainen et al., 2011). Although tibia CSA at birth was significantly larger in children whose mothers had a high vitamin D status during pregnancy (Viljakainen et al., 2010), the differences between groups in mean CSA change over 14 months or in final CSA at 14 months did not reach statistical

significance (Viljakainen et al., 2011). **The Panel** notes that maternal 25(OH)D at or below about 43 nmol/L during pregnancy was associated with bone outcomes in the child at birth, which did not persist at the age of about one year possibly due to infant vitamin D supplementation starting at two weeks of age.

5.1.3.5. Summary of conclusions on serum 25(OH)D concentration and health outcomes in pregnancy

The Panel notes that the evidence on a possible threshold value for serum 25(OH)D concentration with regard to adverse pregnancy-related health outcomes shows a variability of results. Several factors contribute to this (as also discussed in Sections 5.1.1.1.1, 5.1.1.1.3, 5.1.1.1.4. for musculoskeletal health outcomes in adults) and also include the large variation in the results from different laboratories and assays used for measuring serum 25(OH)D concentrations (Section 2.4.1). Furthermore, observational studies often used single measurements of 25(OH)D concentration, thus possible changes in 25(OH)D concentration throughout pregnancy were not considered in the analyses of the relationship with health outcomes.

The Panel concludes that, regarding the relationship between maternal serum 25(OH)D concentration and

- pre-eclampsia, there is inconsistent evidence of an association between maternal serum 25(OH)D concentration and risk of pre-eclampsia or severe pre-eclampsia., but that there is some evidence suggesting an increase in the risk at 25(OH)D concentrations below about 50 nmol/L

- risk of SGA, there is inconsistent evidence of an association of maternal 25(OH)D concentration with an increased risk of SGA, but that there is some evidence suggesting an increase in the risk at 25(OH)D concentrations below about 25–37.5 nmol/L.

- risk of pre-term birth, there is no evidence of an association.

- indicators of bone health in the child after birth, although maternal 25(OH)D at or below about 43 nmol/L during pregnancy was associated with bone outcomes in the child at birth, there is no evidence of an association persisting at the age of about one year.

5.1.4. Serum 25(OH)D concentration and health outcomes in lactation

IOM (2011) (Section 4 and Appendix B) noted that, maternal serum 25(OH)D concentrations increased after vitamin D supplementation of lactating mothers, but that this supplementation had no significant effect on either infant serum 25(OH)D concentrations (for supplementation below 100 µg/day) or infant weight or height. The IOM also noted that there was a lack of association between maternal 25(OH)D concentration and maternal post partum changes in BMD, or breast milk calcium content. The IOM considered that neither maternal BMD nor maternal or fetal serum 25(OH)D concentrations could be used to set reference values for vitamin D during lactation.

SACN (2015) considered one review on vitamin D supplementation during lactation in relation to breast milk vitamin D concentration and serum 25(OH)D concentration in exclusively breast-fed infants (Thiele et al., 2013) and stated that the vitamin D concentration of breast milk increased significantly following supplemental vitamin D of ≥ 50 µg/day but not of 10 µg/day.

The Panel undertook a literature search to identify primary studies (RCTs and prospective or case-control observational studies) on the relationship between maternal serum 25(OH)D and health outcomes of mother during lactation, published after the evidence reviewed by IOM (2011). The

Panel also considered the systematic review by Newberry et al. (2014). In its search, as for pregnancy-related outcomes (Section 5.1.2.), the Panel did not consider data on lactating adolescent. The Panel identified one study published in 2010 on the relationship between maternal serum 25(OH)D and health outcomes of lactating women that is described hereafter.

Salama and El-Sakka (2010) assessed vitamin D in a cohort of 32 breastfed infants (exclusively (n = 20) or partially) with rickets (including nine with hypocalcaemic seizures) and their lactating mothers, in Egypt. Subjects were identified based on clinical presentation, biochemical results and radiological findings, and serum concentrations of calcium, phosphorus, ALP, 25(OH)D and PTH were measured (calcium intake was not reported). Neither infants or their mothers received calcium or vitamin D supplementation and all had limited sun exposure. Infants were aged (mean ± SD) 3.7 ± 1.6 months or 12.4 ± 4.3 months, in the groups with or without hypocalcaemic seizures, respectively. Median (IQR) serum 25(OH)D concentration was 40 (45) nmol/L in mothers (range 10–175 nmol/L), and was 37.5 (32.5) nmol/L in infants (range: 7.5–95 nmol/L), with median (IQR) of 17 (25) and 45 (25) nmol/L in the groups with or without hypocalcaemic seizures, respectively. The correlation between serum 25(OH)D concentrations in rachitic infants and serum 25(OH)D concentrations in their mothers (r = 0.326) was not statistically significant. **The Panel notes** that this study found no significant association between serum 25(OH)D concentrations in infants with rickets and in their mothers.

Conclusions on serum 25(OH)D concentration and health outcomes in lactation

The Panel notes that the only recent study identified by the Panel found no significant association between serum 25(OH)D concentrations in infants with rickets and serum 25(OH)D concentrations in their mothers. Data on the low concentration of vitamin D in breast milk, and on vitamin D intake and status of lactating women were discussed by the Panel previously (Section 2.3.7.2.).

The Panel concludes that there is no evidence for a relationship between serum 25(OH)D concentration and health outcomes of lactating women that may be used to set a DRV for vitamin D.

5.1.5. Serum 25(OH)D concentration and non-musculoskeletal health outcomes

For non-musculoskeletal health outcomes, as indicated in the introduction of Section 5.1., the Panel considered the evidence collated in and conclusions of the report by IOM (2011), the systematic review by Newberry et al. (2014) and the draft report by SACN (2015). The Panel's main objective in this section was to investigate whether data on serum 25(OH)D concentration and non-musculoskeletal health outcomes may be used to set a target value for serum 25(OH)D in order to derive DRVs for vitamin D. As the three reports the Panel considered may have had different objectives (e.g. without always drawing separate conclusions for vitamin D intake and vitamin D status), the overall conclusions of these reports with regard to the relationship between vitamin D intake (either alone or with calcium) or status (i.e. serum 25(OH)D concentration) and several health outcomes are briefly summarised below.

The three reports covered often the same health outcomes (cancer, cardiovascular diseases (CVD), markers of immune function, function of the nervous system and risk of related disorders, non-skeletal obstetric outcomes), with some exceptions. For example, all-cause mortality and pancreatic cancer were covered by Newberry et al. (2014) and not by IOM. Type 2 diabetes and metabolic syndrome, functions of the nervous system and risk of related disorders (e.g. cognition, mood, depression, autism) and non-skeletal obstetric outcomes were covered by IOM (2011) (Appendix B) and not by Newberry et al. (2014). Other cancers (such as oesophagus, stomach cancer, larynx, oropharynx, lung, endometrium, ovary, kidney, non-Hodgkin, liver, bladder cancer, melanoma and basal cell skin cancer and melanoma), maternal serum 25(OH)D concentration in pregnancy and

later cognitive and psychological development of the offspring, neonatal hypocalcaemia, oral health and age-related macular degeneration (AMD) were only covered by SACN (2015).

According to these reports, there is no or an inconsistent relationship between vitamin D intake (with or without calcium) or status and all-cause mortality or total cancer risk and mortality, though SACN (2015) reported conclusion from a systematic review that vitamin D supplementation in combination with calcium reduces mortality risk and that this is not seen with vitamin D supplementation alone. Most of the evidence on breast cancer, colorectal cancer and prostate cancer, was of observational nature and was considered of limited value or inconsistent or insufficient to conclude on a dose-response relationship. However, Newberry et al. (2014) concluded that the only observational evidence identified in their update for pancreatic cancer found an increase in the risk with increased serum 25(OH)D concentration.

For total CVD/cardiovascular events and hypertension, IOM (2011), Newberry et al. (2014) and SACN (2015) concluded that no or an inconsistent relationship was found between vitamin D intake (with or without calcium) or status and the risk of these outcomes, based on evidence which was considered limited, not statistically significant or not supported by intervention studies. However, when addressing CVD mortality separately, Newberry et al. (2014) concluded that 8 observational studies (prospective cohort or nested case-control studies, no RCTs) showed a higher risk for cardiovascular death for subjects with the lowest serum 25(OH)D concentrations (lower bounds throughout all the studies ranged between 8 and 40 nmol/L) compared to those with the highest (higher bounds ranged between 45 and > 100 nmol/L).

The evidence on type 2 diabetes and metabolic syndrome (obesity) was considered not conclusive by the IOM for the purpose of setting DRVs. In addition, limited or inconsistent evidence of mostly observational nature was also found on the relationship between vitamin D intake (either alone or with calcium) or status and functions of the nervous system and the risk of related disorders.

For markers of immune function, IOM (2011), Newberry et al. (2014) and SACN (2015) considered a variety of outcomes including asthma, autoimmune diseases, wheeze, atopy and various infectious diseases and the IOM and the SACN concluded that the evidence for a cause and effect relationship was insufficient for setting DRVs for vitamin D.

For non-skeletal obstetric outcomes (caesarean section, obstructed labour in the mother, and immune-related outcomes in the offspring such as type 1 diabetes mellitus, asthma and atopic eczema, or other outcomes in the offspring e.g. Apgar score), the IOM and the SACN concluded that the evidence is limited and not conclusive, as conflicting results are shown in observational studies and RCTs.

For all the health outcomes (other cancers, maternal serum 25(OH)D concentration in pregnancy and later cognitive and psychological development of the offspring, neonatal hypocalcaemia, oral health, AMD) assessed only by SACN (2015), the evidence from observational studies is not supported by robust clinical trials or evidence is lacking, or inconsistent, or only weak.

The Panel considers that the available evidence on these non-musculoskeletal-related health outcomes is insufficient to be used as criteria for setting DRVs for vitamin D.

5.1.6. Overall conclusions on serum 25(OH)D concentration and various health outcomes, in relation to the setting of DRVs for vitamin D

The Panel notes that most evidence on the relationship between serum 25(OH)D concentration and health outcomes is related to musculoskeletal health outcomes (Section 5.1.1.). The Panel notes that the evidence on a possible threshold value for serum 25(OH)D concentration with regard to adverse musculoskeletal or pregnancy-related health outcomes, that may be used to inform the setting of

DRVs for vitamin D, shows a wide variability of results (Sections 5.1.1.1.7., 5.1.1.2.4. and 5.1.2.). Several factors contribute to this (Sections 5.1.1.1.1, 5.1.1.1.3, 5.1.1.1.4.) and also include the large variation in the results from different laboratories and assays used for measuring serum 25(OH)D concentrations (Section 2.4.1). Furthermore, observational studies mostly used single measurements of 25(OH)D concentration, thus possible long-term changes in 25(OH)D concentration were not considered in the analyses of the relationship with health outcomes.

Taking into account the overall evidence and uncertainties for adults (Section 5.1.1.1.5.) and infants and children (Section 5.1.1.2.4), the Panel considers that there is sufficient evidence for an increased risk of adverse musculoskeletal health outcomes at 25(OH)D concentration below 50 nmol/L. Taking into account the overall evidence and uncertainties for pregnancy (Section 5.1.2.), the Panel considers that there is also evidence for an increased risk of adverse pregnancy-related health outcomes at 25(OH)D concentration below 50 nmol/L. **The Panel concludes that this concentration can be used as a target value to derive a DRV for vitamin D intake for adults, infants, children and pregnant women**. The setting and analyses of the available studies do not allow a conclusion to be drawn as to whether this concentration should be achieved by about half of or most subjects in the population.

The Panel notes that there is no evidence for a relationship between serum 25(OH)D concentration and health outcomes of lactating women that may be used to set a DRV for vitamin D.

5.2. Vitamin D intake from supplements and musculoskeletal health outcomes, pregnancy and lactation

Following a similar approach as in Section 5.1. for serum 25(OH)D concentration and health outcomes, the Panel considered studies (here, preferably RCTs) on vitamin D intake (mostly as supplements, with or without calcium) and various health outcomes (several musculoskeletal health outcomes, health outcomes in pregnancy and lactation, as defined in Section 5.1.), to evaluate whether they might inform the setting of DRVs for vitamin D.

5.2.1. Bone mineral density/content in adults

IOM (2011) (Sections 4 and 5.1.1.1.1., Appendix B) reported that most of the studies (all expect one of the 18 RCTs cited) evaluated the effect of vitamin D supplementation in combination calcium supplementation, often without information on the habitual dietary intakes from foods (eight RCTs). These RCTs were predominantly conducted in postmenopausal women, using supplemental vitamin D at doses of 7.5–25 µg/day (all expect two RCTs), along with 377-1,450 mg/day of calcium. From these RCTs, the IOM concluded that there was evidence that supplementation of vitamin D plus calcium (compared with placebo) resulted in small increases in BMD of the spine, total body, femoral neck and total hip, but that the evidence on vitamin D supplementation alone and BMD was limited. SACN (2015) (Section 5.1.1.1.1) concluded that the evidence was suggestive of an effect of vitamin D supplementation on bone health indices at some skeletal sites in adults aged > 50 years, but that the evidence for adults < 50 years was inconsistent or insufficient to draw conclusions.

The Panel takes into account the same six RCTs that were considered in relation to associations between serum 25(OH)D concentrations and BMD/BMC, from which only one (Macdonald et al., 2013) provided data on vitamin D intake from food and supplements other than that of the intervention in the study population (Section 5.1.1.1.1.). The Panel notes that two of the six RCTs found no effect on BMD of vitamin D plus calcium, from supplements or fortified foods, at doses of about 71 µg/day (Jorde et al., 2010) or 20 µg/day (Kukuljan et al., 2011), in subjects with mean baseline concentrations of 58 and 86 nmol/L, respectively.

In contrast, three RCTs (Section 5.1.1.1.1.) in subjects with mean baseline concentrations of 25(OH)D of 34–50 nmol/L reported an increase in BMD or a decrease in BMD loss following vitamin D supplementation at doses of 10–25 µg/day (with or without calcium) (Islam et al., 2010; Kärkkäinen et al., 2010; Macdonald et al., 2013) (results from unadjusted analyses in (Kärkkäinen et al., 2010)). One RCT (Nieves et al., 2012) in subjects with mean baseline concentration of 29 nmol/L found an increase in BMD following vitamin D supplementation with 25 µg/day plus calcium only in subjects with the *FF* genotype (but not in subjects with the *Ff/ff Fok1* genotypes). The controls (to which the intervention was compared to) in these studies were of various nature (Section 5.1.1.1.1.).

For the present Section, the Panel also identified one prospective observational study in 9,382 women and men in Canada aged 25 years to more than 71 years and followed for 10 years, that investigated changes over time in calcium and vitamin D intakes (from foods and supplements, assessed repeatedly by FFQs), and their longitudinal associations with BMD (Zhou et al., 2013). The Panel notes that, in this study, after adjustments for potential confounders, vitamin D intakes ≥ 10 µg/day (mean of the 10-year) were positively associated with 10-year BMD change at total hip or femoral neck, compared with intakes of vitamin D < 5 µg/day, in women (but not in men) (e.g. for total hip: 0.008 g/cm^2; 95% CI: 0.003–0.013).

The Panel notes that the results of these studies with heterogeneous designs are not consistent. In line with the conclusions of the report by IOM (2011), altogether, the Panel notes that there is some evidence suggesting that beneficial effects of vitamin D supplementation on BMD/BMC may be achieved with doses of about 10 to 25 µg/day in non-institutionalised subjects with 25(OH)D concentrations between 25 and 50 nmol/L, and that the effects may depend on calcium intake.

5.2.2. Fracture risk in adults

IOM (2011) (Sections 4 and 5.1.1.1.3., Appendix B) reviewed a total of 19 RCTs identified by Cranney et al. (2007) (15 RCTs), Chung et al. (2009) (two RCTs) or by additional literature searches (2 RCTs). These RCTs provided vitamin D_2 or D_3 (with or without calcium), with various doses (e.g. out of the 15 RCTs identified by Cranney et al. (2007), 11 used vitamin D_3 doses of 7.5-20 µg/day), at various frequency (e.g. daily, every four months, once per year), and often with no information on the habitual dietary intake of vitamin D from foods. The IOM concluded that vitamin D supplementation with calcium was effective in reducing fracture risk (total or hip) in institutionalised older populations only (considering a limited number of studies out of the 15 RCTs identified by Cranney et al. (2007)), but that the evidence for a benefit of vitamin D and calcium supplementation on fracture risk in community-dwelling individuals was inconsistent across trials.

Newberry et al. (2014) identified one RCT using vitamin D and calcium, that assessed fracture risk, and that was not already considered by the IOM. This RCT (Prentice et al., 2013) was a re-analysis of data from a previous trial that attempted to assess the effects of daily supplementation with 10 µg vitamin D and 1,000 mg calcium, consumed over an average intervention period of seven years (habitual dietary intake not reported). Results were provided for the whole study group as well as for those that were not using personal supplements at baseline. The study found no significant effect of the intervention on overall total fracture risk.

SACN (2015) identified one RCT already considered by the IOM and that used a single high annual dose of vitamin D (Sanders et al., 2010), reported mixed evidence from three meta-analyses on vitamin D supplementation and fracture prevention, and concluded that evidence from RCTs do not show an effect of vitamin D supplements on fracture risk in older men and women. One meta-analysis of 19 RCTs was supportive of a beneficial effect of vitamin D supplementation (D_2 or D_3, with or without calcium) of doses above 10 µg/day in reducing the risk of non-vertebral fractures (9 RCTs) and hip fractures (5 RCTs) (Bischoff-Ferrari et al., 2009b). In contrast, the two other

meta-analyses (of 53 and 12 RCTs, respectively) showed that 'vitamin D' alone had no effect on fracture risk, contrary to vitamin D plus calcium (Avenell et al., 2014; Bolland et al., 2014). However, Avenell et al. (2014) did not exclude studies using supplementation with vitamin D metabolites and only Bischoff-Ferrari et al. (2009b) included exclusively studies based on oral supplementation (12 on oral vitamin D_2 or D_3 out of 19 RCTs included). All three systematic reviews included studies on institutionalised subjects; few included studies were published in 2010 or afterwards (two in Bolland et al. (2014) and five in Avenell et al. (2014)) i.e. after the IOM report; and several studies were in common in these three reviews. The Panel considers that no conclusion can be drawn from these systematic reviews for the setting of DRVs for vitamin D.

For the present Section, the Panel considered a population-based Swedish cohort, which included 61,433 women (born between 1917 and 1948, mean ages of quintiles between 56 and 59 years) followed for 19 years (Snellman et al., 2014). Total dietary intakes (from foods and supplements) were assessed repeatedly by several FFQs. Women with a total intake higher than 12.5 µg/day did not have a lower rate of fracture of any type, compared with those with a total vitamin D intake below 3.5 µg/day. Calcium intake (higher or less than 800 mg/day) did not modify these results. The Panel notes that, in this study, dietary intakes of vitamin D, from foods and supplements, was not associated with the rate of fractures in community-dwelling middle-aged women.

The Panel notes that the available evidence does not indicate that, in community-dwelling adults with adequate calcium intakes, vitamin D supplementation up to 20 µg/day has a significant positive effect on fracture risk.

5.2.3. Muscle strength/function and physical performance in adults

IOM (2011) (Sections 4, 5.1.1.1.4. and Appendix B) noted that randomised trials suggest that vitamin D dosages of at least 20 µg/day, with or without calcium, may improve physical performance measures, but that the evidence was insufficient to define the shape of the dose–response curve. The findings by Lamberg-Allardt et al. (2013) and Newberry et al. (2014) have been described previously (Section 5.1.1.1.4.).

The Panel takes into account the same seven RCTs with heterogeneous designs, which were considered in relation to associations between serum 25(OH)D concentrations and muscle strength/function and physical performance. From these, only one provided data on habitual dietary intake of vitamin D (means of 1.6 and 4.1 µg/day in the placebo and intervention groups, respectively (Pirotta et al., 2015) (Section 5.1.1.1.4.). Overall, these RCTs do not provide evidence for an effect of vitamin D supplementation (10 to about 71 µg/day), with or without calcium, on these outcomes. However, one study showed a beneficial effect of vitamin D supplementation (vs placebo) on postural stability in the subgroup of subjects with elevated baseline body sway (Lips et al., 2010). Another one showed a beneficial effect of vitamin D supplementation with calcium (vs calcium) on muscle strength and mobility in those who were the weakest and slowest at baseline (Zhu et al., 2010). A third one found a beneficial effect of vitamin D supplementation (two different doses) on the ability to do chair-stand tests in subjects with the slowest gait speed at baseline (Lagari et al., 2013). These three studies used doses ranging between 10 and 50 µg/day.

For the present Section, the Panel also identified a double-blind RCT in 305 'healthy' postmenopausal women (aged 60-70 years; BMI 18-45 kg/m^2) in Scotland, receiving vitamin D_3 supplementation of 10 and 25 µg/day or placebo for one year and the effects on grip strength (Wood et al., 2014). The Panel notes that supplementation had no effect on grip strength in these women, with a mean baseline serum 25(OH)D concentration of around 33 nmol/L and median habitual dietary intake of vitamin D of about 4.3–4.8 µg/day.

The Panel notes that these studies suggest that vitamin D supplementation does not generally affect muscle strength/function and indices of physical performance. However, sub-group analyses on small numbers of older subjects, with impaired indices of physical performance at baseline, indicated beneficial effects of vitamin D supplementation doses (ranging between 10 and 50 µg/day) in three of these studies.

5.2.4. Risk of falls and falling in adults

IOM (2011) (Sections 4, 5.1.1.1.5. and Appendix B) concluded, based on Cranney et al. (2007) and Chung et al. (2009) and additional literature search, that, some RCTs found a significant effect of vitamin D supplementation on fall incidence or risk or number of fallers, but the greater part of the 20 RCTs considered found no effect of supplemental vitamin D (usually with doses of 10-20 µg/day and 50 µg/day in one), with or without supplemental calcium, on the risk of falls. A number of RCTs analysed falls rather than fallers.

Newberry et al. (2014) identified two RCTs that examined the effect of supplementation with vitamin D and calcium on the risk of falls/falling among community-dwelling older adults (Prince et al., 2008; Pfeifer et al., 2009) considered by IOM (2011). Prince et al. (2008) supplemented older women daily with 25 µg vitamin D_2 and 1,000 mg calcium or only 1,000 mg calcium in a one-year RCT and found a significantly decreased risk of falling at least once, and a decreased risk for first falls, especially in winter/spring. In the one-year RCT performed by Pfeifer et al. (2009), older individuals received daily either 20 µg vitamin D_3 and 1,000 mg calcium or only 1,000 mg calcium and found a reduction in the number of first fallers in the group that received vitamin D_3.

The Panel also notes the above mentioned RCT (Section 5.2.3.) by Wood et al. (2014) that showed no effect of vitamin D_3 supplementation (10 or 25 µg/day versus placebo) on the number of 'ever fallen' falls in healthy post-menopausal women.

The Panel considers that, among studies identified by IOM (2011) and Newberry et al. (2014), some provide evidence of an effect on falls or the number of fallers with daily 20–25 µg vitamin D_2/D_3 with calcium in comparison with calcium alone in community-dwelling older adults, whereas one RCT retrieved by the Panel thereafter in healthy postmenopausal women did not find such effect of vitamin D_3 compared with placebo.

5.2.5. Bone mineral density/content in infants and children

For infants, **IOM (2011)** identified two RCTs (Greer et al., 1982; Greer and Marshall, 1989), using supplemental doses of 10 µg/day vitamin D, and which found inconsistent effects on BMC (Sections 4, 5.1.1.2.1. and Appendix B).

The Panel takes into account the same two randomized trials (Holmlund-Suila et al., 2012; Gallo et al., 2013) that were considered in relation to associations between serum 25(OH)D concentrations and BMD/BMC (Section 5.1.1.2.1.). They used various doses of vitamin D_3 supplementation, without a placebo group, in (mostly) breastfed infants. Only one provided data on the vitamin D intake through breast milk between ages 1 and 12 months (1–6 µg/day) (Gallo et al., 2013). They showed that a supplementation with 10 µg/day vitamin D_3 was sufficient to reach a plasma/serum 25(OH)D of at least 50 nmol/L in (almost) all subjects, and that there was no significant differences in several bone measurements between groups.

For children, **IOM (2011)** considered five RCTs (Ala-Houhala et al., 1988b; Cheng et al., 2005; El-Hajj Fuleihan et al., 2006; Viljakainen et al., 2006b; Andersen et al., 2008) performed in children of various ages and receiving doses of vitamin D between 5 and about 50 µg/day (Sections 4, 5.1.1.2.1. and Appendix B). Only three of them provided data on habitual dietary intake of vitamin D. Three

studies did not find an effect of these doses on BMC/BMD, while one study found an effect with 5 and 10 µg/day only in subjects with compliance above 80 % (but not in the ITT analysis) and another with 50 µg/day.

The Panel takes into account the same RCT that was considered in relation to associations between serum 25(OH)D concentrations and BMD/BMC (Section 5.1.1.2.1.). Molgaard et al. (2010) supplemented 12 year-old girls with either placebo, 5 or 10 µg vitamin D/day for one year, in addition to the habitual dietary intake of vitamin D (mean intakes of 2.6, 2.8 and 2.5 µg/day, respectively) and found no effect on BMC/BMD.

The Panel notes that the data available on vitamin D supplementation in infants (10 µg/day or higher) and children (5 to 50 µg/day) and BMD/BMC are inconsistent. The Panel however notes that two recent trials showed that a supplementation with 10 µg/day vitamin D_3 in (mostly) breastfed infants was sufficient to reach a plasma/serum 25(OH)D of at least 50 nmol/L in (almost) all subjects.

5.2.6. Pregnancy, lactation and related outcomes in mothers and infants

For pregnancy, **IOM** (Sections 4, 5.1.2., 5.1.3. and Appendix B) considered one RCT that found no effect of maternal vitamin D supplementation in combination with calcium on the incidence of preeclampsia (Marya et al., 1987), and reported on four RCTs that found no effect of maternal vitamin D supplementation, on birth weight or length of the children (Brooke et al., 1980; Maxwell et al., 1981; Mallet et al., 1986; Marya et al., 1988). In these studies, the supplementation was generally based on doses of 25-30 µg/day, and started at various timepoints in pregnancy.

The Panel takes into account the same paper by Wagner et al. (2013b) that was considered in relation to associations between serum 25(OH)D concentrations and health outcomes in pregnancy (Section 5.1.2.). This paper reported on pooled data from two RCTs in which daily supplementation doses of 50 and 100 µg vitamin D_3 during pregnancy had no effect on neonatal birth weight, and risk of pre-eclampsia or preterm birth in pregnant women with mean serum 25(OH)D concentrations of 57–65 nmol/L at baseline. The Panel did not retrieve any relevant RCT on vitamin D intake/supplementation during lactation and relevant outcomes in mother or child.

The Panel notes that the number of RCTs, that focused on effects of supplementation during pregnancy or lactation on outcomes related to e.g. bone, pre-eclampsia and birth weight, is small. The doses used in the few studies reported varies between 25 and 100 µg/day, with no effect on the variables studied. In addition, the amount of vitamin D in human milk is modestly correlated with maternal vitamin D intake up (unless high supplemental doses are used) (Section 2.3.7.).

5.2.7. Overall conclusions on vitamin D intake from supplements and musculoskeletal health outcomes, pregnancy and lactation, in relation to the setting of DRVs for vitamin D

The Panel concludes that:

- there is some evidence suggesting that beneficial effects of vitamin D supplementation on BMD/BMC may be achieved with doses of about 10 to 25 µg/day in non-institutionalised subjects with 25(OH)D concentrations between 25 and 50 nmol/L, and that the effects may depend on calcium intake,

- available studies suggest that vitamin D supplementation does not generally affect muscle strength/function and indices of physical performance. However, sub-group analyses on small numbers of older subjects, with impaired indices of physical performance at baseline,

indicated beneficial effects of vitamin D supplementation doses (ranging between 10 and 50 µg/day) in three studies,

- although results of available studies on vitamin D supplementation with or without calcium are not entirely consistent, there is some evidence for an effect on the risk of falls/falling with daily 20-25 µg vitamin D supplementation with calcium in comparison with calcium alone, in community-dwelling older subjects,

- available studies provide no evidence for an effect of vitamin D supplementation on fracture risk,

- the available data do not allow conclusion to be drawn on an effect of vitamin D supplementation on BMD/BMC in infants and children. However, two recent trials showed that a supplementation with 10 µg/day vitamin D_3 in (mostly) breastfed infants was sufficient to reach a plasma/serum 25(OH)D of at least 50 nmol/L in (almost) all subjects,

- available studies provide no evidence for an effect of vitamin D supplementation on a number of outcomes in pregnancy or lactation.

Overall, the Panel notes that there may be beneficial effect of vitamin D supplementation above 10 µg/day (in addition to the habitual dietary intake of vitamin D) on some musculoskeletal health outcomes, particularly in subjects with compromised musculoskeletal health or 'low' 25(OH)D concentration. Habitual dietary intake of vitamin D is generally low (Section 3.2.); however, the Panel notes that, in these supplementation studies with heterogeneous designs, vitamin D intake from foods was reported only in a limited number of trials. In addition, the extent to which cutaneous vitamin D synthesis has contributed to the vitamin D supply, and thus may have confounded the relationship between vitamin D intake and reported outcomes, is not known. The Panel concludes that these data are not useful as such for setting DRVs for vitamin D. For the purpose of deriving DRVs for vitamin D, these data may only be used to support the outcome of the characterisation of the vitamin D intake-status relationship undertaken by the Panel under conditions of minimal endogenous vitamin D synthesis (Section 5.3.).

5.3. Vitamin D intake and serum 25(OH)D concentration

The relationship between vitamin D intake and serum 25(OH)D concentrations has been investigated in numerous intervention studies in all age groups including different doses of vitamin D provided as supplements or as foods or fortified foods.

The systematic reviews by Cranney et al. (2007) and Chung et al. (2009), which were used by **IOM (2011),** included RCTs using supplements or fortified foods. Focusing on 28 RCTs (26 on adults), Chung et al. (2009) concluded that a relationship between increasing supplementation doses of vitamin D_3 and increasing net change in serum 25(OH)D concentration was evident in both adults and children, that the dose-response relationships differed depending on serum 25(OH)D concentration of the participants at baseline (< 40 nmol/L vs > 40 nmol/L), and depending on the duration of supplementation (< three months vs > three months). The range of supplementation doses was large (5-125 µg/day), the baseline serum 25(OH)D concentrations varied and the assays used for measuring serum 25(OH)D concentrations were heterogeneous. Supplementation with vitamin D_2 was more commonly used than supplementation with vitamin D_3 in RCTs in infants and pregnant or lactating women, with a resulting significant increase in serum 25(OH)D concentrations in infants or lactating mothers and in cord blood. Based on Cranney et al. (2007) and Chung et al. (2009) and some new RCTs, IOM (2011) undertook specific **meta-regression** analyses to obtain a dose-response curve, in order to set DRVs for vitamin D (Section 5.3.1.).

Lamberg-Allardt et al. (2013) considered the results from four systematic reviews (Cranney et al., 2007; Chung et al., 2009; Cashman et al., 2011b; Black et al., 2012) (Section 5.3.1. for Cashman et al. (2011b)) on the relationship between vitamin D supplementation/fortification and serum 25(OH)D concentrations, and underlined the important issue of the heterogeneity in the results according to the assays used to measure serum 25(OH)D concentrations. Lamberg-Allardt et al. (2013) concluded that the systematic reviews indicated a clear effect of supplementation and fortified foods on the serum 25(OH)D concentration, but the doses needed to achieve specific concentrations of 25(OH)D are difficult to determine. One systematic review (Black et al., 2012) estimated that 1 µg vitamin D ingested only from fortified foods increased the serum 25(OH)D concentration by 1.2 nmol/L (heterogeneity index (I^2) = 89%, adjusted R^2 = 0.67). Habitual dietary intake of vitamin D was usually not reported in the 16 RCTs included in this review thus was not added to the content of the fortified foods for the data analysis.

Newberry et al. (2014) identified one systematic review (Autier et al., 2012) that included 76 placebo-controlled and open-label trials published from 1984 through 2011 and addressed the relationship between supplementation with vitamin D_2 or D_3 (oral or injection, with or without calcium, with vitamin D doses ranging from 5 to 250 µg/day (median : 20 µg/day)) and net change in serum 25(OH)D concentrations. The **meta-regression** analysis by Autier et al. (2012) of serum 25(OH)D concentration on (log-transformed) vitamin D doses (less than 100 µg/day) showed that serum 25(OH)D concentrations increased by an average of 1.95 nmol/L for each 1 µg per day vitamin D_3 supplementation (without calcium). In this analysis, vitamin D_2 supplementation resulted in smaller increases compared with vitamin D_3 supplementation, and simultaneous supplementation with calcium resulted in non-significantly smaller increases in serum 25(OH)D concentrations. As the number of trials that used higher doses of vitamin D was small (n = 3 with doses of 100 µg/day or more), whether the dose-response relationship reaches a plateau at higher doses could not be assessed. Newberry et al. (2014) noted that most studies included in (Autier et al., 2012) did not stratify findings by sex, and the review itself did not stratify findings by assay method. In addition to the systematic review by Autier et al. (2012), Newberry et al. (2014) identified eighteen new RCTs (in addition to those included by Chung et al. (2009)) (two of them using fortified foods, the others using vitamin D supplements with or without calcium, one study using vitamin D_2 supplement). Overall, all studies reported an increase in serum 25(OH)D with vitamin D supplementation. Newberry et al. (2014) also provided plots showing the relationship between vitamin D_3 supplementation doses and net changes in serum 25(OH)D concentrations in 44 RCTs, according to populations (adults and children), baseline serum 25(OH)D concentrations, duration of supplementation, and assay used to assess serum 25(OH)D concentration.

The Panel notes that studies based on vitamin D supplementation and/or food and food fortification suggest a relationship between vitamin D intake and serum 25(OH)D concentrations in all ages and that the effects of the relationship depends on several factors, including baseline serum 25(OH)D concentrations, supplementation dose, study duration, and assay used to assess serum 25(OH)D concentration.

5.3.1. Characterisation of the intake-status relationship in previous approaches

One approach to assess the intake-status relationship could be to rely on a sample of **individual data from a particular study** (e.g. regression analysis on individual data). The Panel did not have access to a sufficiently large and representative sample of individual data from a study considered relevant for the aim of setting DRVs at the European level.

Several bodies have characterised the intake-status relationship through **meta-regression approaches**, which has also been the target of various authors (e.g. (Cashman et al., 2011b; Autier et al., 2012)). In a meta-regression approach, a quantitative synthesis of the dose-response relationship between mean results at group level from studies is usually carried out (taking into

account potential confounders by relevant adjustments). Once the methodological heterogeneity is characterised, the remaining variation reflects a real phenomenon that describes the extent to which different populations behave differently. One advantage of the meta-regression approach is the *representativity*, by considering several studies from various populations in different contexts, instead of relying on specific data from one specific study undertaken in a particular context. However, by using group *means* from studies, the information on the variability between individuals is diminished, which may complicate the setting of e.g. a reference value that would correspond to the intake needed to cover the requirements of 97.5% of *individuals*. The confidence interval (CI) in meta-regression analyses provides an estimate of the uncertainty about the fitted response line due to sampling, but does not provide an estimate of the variability between individuals (Section 5.3.2.).

IOM (2011) carried out **meta-regression analyses** of the relationship between serum 25(OH)D concentrations and log-transformed (Ln) **total intake of vitamin D** (from food and supplements) during winter at latitudes above 49.5°N in Europe or Antarctica, separately **for children/adolescents, young/middle-aged adults, and older adults** (Ala-Houhala et al., 1988b; Van Der Klis et al., 1996; Schou et al., 2003; Larsen et al., 2004; Viljakainen et al., 2006b; Cashman et al., 2008; Cashman et al., 2009; Smith et al., 2009; Viljakainen et al., 2009)[27]. The IOM considered that the response of serum 25(OH)D concentration to vitamin D intake is non-linear, the rise being steeper below 25 µg/day and flattening above 25 µg/day. Baseline serum 25(OH)D concentrations and age did not have a significant effect in the response of serum 25(OH)D concentration to total vitamin D intake. The IOM performed also a **meta-regression analysis on all age-groups** (6 to more than 60 years) at latitudes above 49.5°N using the CI around the mean. The IOM performed as well a separate analysis for latitudes 40–49°N during winter. In particular, this analysis (i) showed that the achieved serum 25(OH)D concentration at these lower latitudes was greater (24%) for a given total intake compared to that achieved in the previous analysis at higher latitudes, and (ii) explained less variability than the model at higher latitudes. Thus, the IOM decided to focus on latitude above 49.5°N to set DRVs for vitamin D. The IOM noted that, at a total intake of 10 µg/day, the predicted mean serum 25(OH)D concentration was 59 nmol/L in children and adolescents, young and middle-aged adults, and older adults (with a lower limit of the CI of about 52 nmol/L). The IOM also noted that, at a total intake of 15 µg/day, the predicted mean serum 25(OH)D concentration was 63 nmol/L (lower limit of the CI of 56 nmol/L). These results were used to set the EAR and RDA for vitamin D, which take into account the uncertainties in these analyses (Section 4).

Cashman et al. (2011b) applied a **meta-regression approach** using different model constructs (curvilinear as in the approach by the IOM, or linear) to explore the most appropriate model of the relationship between total vitamin D intake (from food and supplements) and serum 25(OH)D concentration. Priority was given to data from winter-based RCTs performed at latitudes 49.5-78°N, using vitamin D_3 supplementation (not vitamin D_2) in children and adults (i.e. excluding infants, pregnant and lactating women) and with a duration of at least six weeks (Harris and Dawson-Hughes, 2002). Thus, n = 12 RCTs in 11 references were included (Ala-Houhala et al., 1988b; Honkanen et al., 1990; Pfeifer et al., 2001; Meier et al., 2004; Barnes et al., 2006; Viljakainen et al., 2006a; Cashman et al., 2008; Cashman et al., 2009; Smith et al., 2009; Viljakainen et al., 2009; Cashman et al., 2011a). When the included RCTs did not assess and/or did not report the habitual vitamin D intake (Ala-Houhala et al., 1988b; Honkanen et al., 1990; Pfeifer et al., 2001; Meier et al., 2004), the authors considered the mean intake of the relevant age and sex group, from the national nutrition survey preferably from the country in which the RCT was preformed. A combined **weighted linear model** meta-regression analysis of log-transformed (Ln) **total vitamin D intake** (maximum 50 µg/day) versus achieved serum 25(OH)D in winter produced a curvilinear relationship. Use of **non-transformed** total vitamin D intake data (maximum 35 µg/day, Section 2.4.1. and (Aloia et al., 2008)) provided a linear relationship. At an intake of 15 µg/day (i.e.

[27] All these studies used vitamin D_3 supplementation.

the RDA set by the IOM for vitamin D for adults aged 19–70 years, Section 4), the predicted serum 25(OH)D concentration at the 95% lower limit of the CI of the log-transformed and the linear models was 54.4 and 55.2 nmol/L, respectively. The total vitamin D intake estimated to achieve the 'RDA-type' and 'EAR-type' values for 25(OH)D concentrations set by the IOM (50 and 40 nmol/L, Section 4) was 9 µg/day for 50 nmol/L (and 2.7 µg/day for 40 nmol/L) in the log-transformed model. In the linear model, this intake was 12 µg/day for 50 nmol/L (and 6.5 µg/day for 40 nmol/L), respectively. In further publications of the same author, use of a 95% prediction interval (PI) in meta-regression analyses was considered to allow for estimation of the requirement of 97.5% of the population (Cashman and Kiely, 2014; Cashman, 2015).

The Nordic Council of Ministers (2014) performed two meta-regression analyses of $\log_{10}$ (serum 25(OH)D) versus total vitamin D intake. The included studies were selected mainly from the systematic review by Cashman et al. (2011b) and the previous Nordic recommendations (NNR, 2004), and studies using doses of vitamin D higher than 30 µg/day were excluded. The **first meta-regression analysis** included six supplementation studies pertinent to the Nordic countries, undertaken in **adults (≤ 60 years)** (Barnes et al., 2006; Cashman et al., 2008; Viljakainen et al., 2009) **and children** (Ala-Houhala et al., 1988b; Molgaard et al., 2010; Cashman et al., 2011a), during winter, at latitudes 50–61°N. The response to intake was found to be limited or absent for baseline concentrations above 50 nmol/L. It was considered that an intake of 7.2 µg/day would maintain a mean serum concentration during winter of about 50 nmol/L for 50% of subjects. Using the lower limit of the 95% CI, it was considered that about 10 µg/day would be sufficient for most of the population. The **second meta-regression analysis** was based on supplementation studies in **mainly older adults** (> 65 years) (Sem et al., 1987; Pfeifer et al., 2001; Meier et al., 2004; Viljakainen et al., 2006a; Cashman et al., 2009) during winter at latitudes 51-61°N. It was considered that an intake of about 5 µg/day would maintain a mean serum 25(OH)D concentration of about 50 nmol/L during wintertime. This estimate was lower than for younger adults, but the 95% CI was wider and, based on its lower bound, it was considered that an intake of about 10-11 µg/day is sufficient for most of this population. These results were used to set the reference values for vitamin D in the Nordic Countries (Section 4).

The Panel applied the meta-regression approach to assess the intake-status relationship with the aim to set DRVs for vitamin D.

5.3.2. Characterisation of the intake-status relationship by EFSA in adults and children

As indicated previously (Section 2.3.1.), the Panel considered that the association between vitamin D intake and status for the purpose of deriving DRVs for vitamin D should be assessed under conditions of minimal endogenous vitamin D synthesis.

5.3.2.1. Methods

As preparatory work for the setting of DRVs for vitamin D, a comprehensive literature search and review was performed to identify and summarise studies that could be used to assess the dose-response relationship between oral vitamin D_2 or vitamin D_3 intake and plasma/serum 25(OH)D concentration (Brouwer-Brolsma et al., 2016).

Prospective studies (that primarily aimed to investigate the dose-response association of vitamin D intake and status) and trials that investigated vitamin D intake and 25(OH)D concentration, published through July 2014 were systematically searched and reviewed. Studies were eligible for inclusion if they:

- were conducted in humans of all ages,

- investigated oral exposure to vitamin D_2 or vitamin D_3 at least twice a week via diet, supplements or fortified foods and its subsequent effect/association on/with 25(OH)D concentration,

- were performed in a **period of assumed minimal endogenous vitamin D synthesis**, i.e. at latitudes above 40°N from October through April (or below 40°S from April through October)[28]. Additional further selections were also proposed (Brouwer-Brolsma et al., 2016), based on the UV index (UV-index < 3) or a simulation model (Webb, 2006; Webb and Engelsen, 2006) (Section 2.3.1.), but in the end were not applied, as it would have led to a substantial reduction in the number of arms (53% and 86 % of the 83 arms would have been excluded respectively),

- and lasted for at least six weeks (Sections 2.4.1. and 5.3.1.).

More information on the inclusion/exclusion criteria and the selection process can be found in (Brouwer-Brolsma et al., 2016).

Finally, 56 articles matched the eligibility criteria, reporting on data of 65 relevant studies (e.g. one article reporting data in children and in adults was considered as one article reporting data on two studies). The majority of the included studies were trials (n = 57), investigating the effects of supplements, fortified foods or foods naturally rich in vitamin D (fish). Only eight prospective cohort studies fulfilled the inclusion criteria.

Using a meta-analytic approach, EFSA performed quantitative syntheses of the summary data extracted by Brouwer-Brolsma et al. (2016) from the included studies. Data from prospective observational studies identified were analysed but were not included in **the meta-regression dose-response model by EFSA, which was based solely on randomised trials data**.

The 57 trials included in the preparatory literature review represented 141 arms. Of these 141 arms, EFSA excluded 58 from the analysis (Appendix D.A), in particular:

- arms from trials on population groups other than children and adults (i.e. infants, pregnant women, lactating women, as these populations represent particular age and/or physiological conditions and the number of arms were low[29]),

- arms resulting in total intakes exceeding the UL set for adults (EFSA NDA Panel, 2012a) (Section 2.2.2.2.),

- arms in which vitamin D_2 was administered. In view of the conflicting results regarding the potential differences in the biological potencies and catabolism of vitamin D_2 and D_3 (Sections 2.3.2. and 2.3.6.), and the low number of arms using vitamin D_2 (six), this exclusion was considered appropriate by the Panel.

- arms for which methodological and/or statistical inconsistencies were identified.

This left 83 arms from 35 trials in the analysis (Appendix D.B), of which nine arms were on children (age range: 2–17 years).

The continuous outcome, i.e. **plasma/serum 25(OH)D** concentration, was analysed by EFSA using the summary data extracted for each arm in each individual study. Background intake was added by EFSA to the supplemental vitamin D dose to generate **total vitamin D intake** estimates. If the

[28] Based on the protocol by Brouwer-Brolsma et al. (2016).
[29] Two arms on pregnant women, three arms on lactating women, three arms on infants.

habitual vitamin D intake of the cohort(s) within a study was not reported in the papers, surrogates were imputed using the appropriate age- and sex- specific mean vitamin D intake values (from food) from the national nutrition survey relevant to the country in which the study was performed (17 trials) (Appendix C).

Two different models of the dose-response relationship between total vitamin D intake and plasma/serum 25(OH)D concentration were explored (Appendix C): a linear model or a non-linear model (i.e. with the natural logarithm transformation of the total intake). Finally, the Panel decided to **retain the non-linear model** to better describe the dose-response shape and to be able to include results from trials using higher supplemental doses (i.e up to 50 µg/day).

A **number of factors potentially influencing the dose-response relationship** (Section 2) were investigated, in order to select factors to be included in the final model to characterise the high heterogeneity of results across individual trials. These were: total vitamin D intake, baseline serum concentration, study duration (≤ three months versus > three months; or ≤ three months, versus three to six months versus one to two years), latitude (as different categories), assay method (HPLC and LC-MS versus immunoassays; or each analytical method as an individual category), period of study publication, BMI (Section 2.3.5.), co-supplementation with calcium, funding source, age, sex, risk of bias (RoB), assessment of compliance, study start period (as a "proxy" to the temporal trends in assay method use, Section 2.4.1.), and ethnicity (as a "proxy" for skin pigmentation and some lifestyle habits that were usually not reported in the included trials). In particular for ethnicity, the data were missing for almost half of the studies, as this information was not reported in the papers (Appendix C).

5.3.2.2. Results

The meta-regression analysis carried out on the selected arms resulted in two predictive equations of achieved serum 25(OH)D concentration:

y = 23.2 Ln (total vitamin D intake in µg/day) (*equation 1, unadjusted model*)

and

y = 16.3 Ln (total vitamin D intake) + 0.5 mean baseline 25(OH)D - 0.5 latitude + 0.9 study start year - 2.0 HPLC - 4.7 LC-MS + 0.6 CPBA - 6.4 ELISA/nr + 1.3 Other assay + 7.8 compliance not assessed (*equation 2, adjusted* model)

The model corresponding to *equation 2* was adjusted for baseline concentration (continuous), latitude (continuous), study start year (continuous), type of analytical method applied (RIA as 'reference' category for the model, HPLC, LC-MS, CPBA, ELISA/not reported (nr), other[30]), assessment of compliance (yes as 'reference' category for the model, no/unknown)). No interaction terms were introduced.

The 95% CI around the coefficient mentioned above for each variable are given in Table 5, Section 8.9. of Appendix C (e.g. about 14.4–18.2 for the coefficient of about 16.3 obtained for Ln (total vitamin D intake)). The summary data of the included studies are given in Appendix D.B., in particular the mean and SD baseline and achieved serum 25(OH)D concentrations per included arm are given in Table 11 of this Appendix.

[30] Based on the data reported by the contractor. 'Other' covers methods presented as 'enzyme immunoassays', Nichols method, 'chemoluminescence immunoassays', 'immunoenzymetric assay' in the references included by the contractor.

After the inclusion of the final set of covariates, the *adjusted* R^2 (proportion of between-study variance explained) of the final model was 85%, meaning that the fitted factors were able to characterise most of the across-trials variability in response.

The two equations above were used to **predict the achieved mean serum 25(OH)D** concentrations corresponding to total vitamin D intakes of 5, 10, 15, 20, 50, 100 µg/day (Appendix C, Table 6) and to **estimate the total vitamin D intakes** that would achieve serum 25(OH)D concentrations of 50, 40, 30, 25 nmol/L (Appendix C, Table 7).

In the *adjusted* multivariable models, all covariates were set to their *mean* values: mean baseline serum 25(OH)D concentration: 50.7 nmol/L; latitude: 53°N; study start year: 2005; assay – HPLC: 10%; LC-MS: 18%; CPBA: 13%; ELISA: 20%; Other: 8%; compliance not assessed/unknown: 27%. As such the adjusted model predictions can be interpreted as referring to an average ideal population in which the major factors influencing the heterogeneity across different populations have been ruled out. Such a reduction in heterogeneity is reflected in the **narrower PI** as compared to the unadjusted model.

Lower and upper limits of the 95% **CI** and of the 95% **PI** were calculated for both the adjusted and the unadjusted model. In the meta-regression context, where a random-effects approach is applied :

- the *CI* illustrates the *uncertainty about the position of the regression line* (i.e. across-study conditional means);

- the *PI* illustrates the *uncertainty about the true mean effect that would be predicted in a future study*.

As such, it is possible to think of the 95% PI only as an ***approximation*** of the interval that would allow for estimation of the requirements for 95% of *individuals* in the overall population, as 95% PI refers to the population of *mean* responses (not *individual* responses) as analysed in the random-effects model.

The role of **BMI** (Section 2.3.5) was tested and it was not included in the final model as a covariate (Appendix C). **Sex** and **age** were also not included in the final model, as they did not further explain between-study variability once mutually adjusted for all other factors. However, regarding the role of age, a stratified analysis was carried out (Appendix D.B), to quantify the impact of the exclusions of the four trials on children (nine arms) (age range: 2–17 years, nine arms) on the predicted achieved mean serum 25(OH)D concentrations (Appendix C, Table 6) and estimated total vitamin D intakes (Appendix C, Table 7).

- In the restricted dataset of 74 arms on adults only, there was an overall small decrease in all serum estimates (and consequently a small increase in total intakes that would achieve target values). Overall estimates did not substantially change as compared to the full data set including children (appendix D.G). Thus, **the Panel decided to retain the data on children and on adults in the dose-response analysis (Section 6).**

- Children tended to achieve the same mean serum 25(OH)D concentrations as the adults at a lower total intake (Appendix D.G). It was not possible to apply a full adjustment to estimate the values based only on the four children trials, as it would have required a much higher minimum number of 'points' per covariate (at least 10 arms for each included factor). **Instead, values from a model adjusted for mean baseline 25(OH)D concentration were provided.** As such these estimates are **not** directly comparable to the ones in the adjusted model in adults, as they are not adjusted for the same set of covariates. The unadjusted model showed lower average intakes, but estimates were less precise; also the highest dose investigated in the included arms was 10 µg/day, so predictions at higher intakes are

extrapolations from the model. For these reasons results from the models on children data could only be evaluated qualitatively.

A number of *sensitivity analyses* were also carried out by EFSA to evaluate whether the findings were robust to the assumptions made in the systematic review protocol and the analyses (Appendix C), in particular, on the background intake imputation process, on eligibility criteria (e.g. fortified food trials versus supplement trials, cf. Section 2.3.2.); characteristics of participants (e.g. exclusion trials that did not explicitly exclude supplement users, persons with sun holidays, persons using sunbeds/artificial UV-B sources or going on sunny holidays). None of these sensitivity analyses raised serious concerns about the robustness of the overall analysis. In addition, there was no particular indication of *publication bias* as explored on the subset of trials for which the mean difference in response could be estimated (Appendix C).

The Panel considers that the results of this meta-regression analysis can be used to set DRVs for vitamin D. The meta-regression model of serum 25(OH)D response to ln of total vitamin D intake from the adjusted model (n = 83 arms) is shown in Figure 3, as well as in Appendix D.F (for comparison with the unadjusted model).

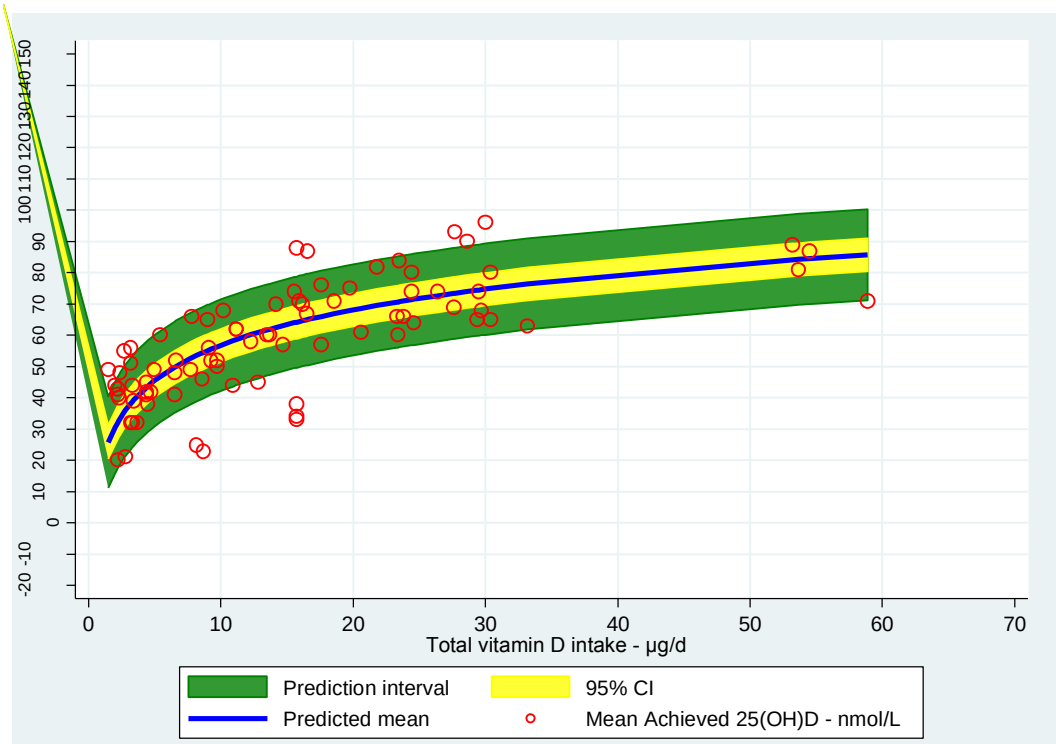

Figure 3: Meta-regression model of serum 25(OH)D response to ln of total vitamin D intake (adjusted model) (n = 83 arms)

5.3.3. Qualitative overview of available data on infants, children, pregnant or lactating women

Only two studies (Ala-Houhala et al., 1986; Atas et al., 2013) that were conducted in breastfed infants met the eligibility criteria of the comprehensive literature search (Brouwer-Brolsma et al., 2016) mentioned previously (Section 5.3.) (in situation of low endogenous vitamin D synthesis). Both studies included an intervention group that was allocated to 10 µg/day vitamin D. Atas et al. (2013) also included a study group that was allocated to 5 µg/day. Ala-Houhala et al. (1986) supplemented with vitamin D_2 for the duration of 15 weeks. At baseline, mean serum 25(OH)D

concentrations were approximately 20 nmol/L, which rose to roughly 80 nmol/L after 15 weeks (values estimated from figures). Atas et al. (2013) supplemented with vitamin D_3 for the duration of 17 weeks, but did not measure baseline serum 25(OH)D concentration. Follow-up measurements at four months of age showed, however, higher serum 25(OH)D concentrations than in the study by Ala-Houhala et al. (1986): serum 25(OH)D reached a median (min-max) level of 99 (43-265) nmol/L in the five µg group, and 141 (80–375) nmol/L in the 10 µg group (Atas et al., 2013).

Three prospective studies (Sullivan et al., 2005; Lehtonen-Veromaa et al., 2008; Andersen et al., 2013) met the eligibility criteria of the comprehensive literature search (Brouwer-Brolsma et al., 2016) mentioned previously (Section 5.3.). Two of these studies reported on dietary vitamin D intake (Sullivan et al., 2005; Lehtonen-Veromaa et al., 2008); one study measured vitamin D intake covering both dietary as well as supplemental intake (Andersen et al., 2013). Vitamin D intakes ranged from median (IQR) 3.9 (1.9–7.0) µg/day ((Andersen et al., 2013), dietary and supplemental intake) to mean of 5.4 ± 1.4 ((Sullivan et al., 2005), dietary intake only). Follow-up time ranged from one (Andersen et al., 2013) to four years (Lehtonen-Veromaa et al., 2008). Mean age at baseline ranged from 11 ± 1 (Sullivan et al. 2005) to 16 ± 2 (Lehtonen-Veromaa et al., 2008) years old. All three studies performed the baseline and follow-up 25(OH)D measurements in February/March. In one study (Andersen et al., 2013), baseline vitamin D intake was (median (IQR)) 3.9 (1.9-7.0) µg/day, food and supplements) and serum 25(OH)D concentrations at follow-up were (median (IQR)) 23 (17–36) nmol/L. For the two others (Sullivan et al., 2005; Lehtonen-Veromaa et al., 2008), baseline vitamin D intakes (food only) were (mean ± SD) 4.0 ± 2.2 and 5.4 ± 1.4 µg/day, while serum 25(OH)D concentrations at follow-up were 48 ± 17 and 50 ± 14 nmol/L.

Two RCTs on pregnant or lactating women met the eligibility criteria of the comprehensive literature search (Brouwer-Brolsma et al., 2016) mentioned previously (Section 5.3.).

In an open-label RCT, Ala-Houhala et al. (1986) examined the effect of vitamin D supplementation on 25(OH)D concentration in pregnant women (41 starters, 39 completers) living in Finland (61°N), delivering in January, and whose age was not reported. Eight women were supplemented with 12.5 µg vitamin D_3 per day throughout the pregnancy; 33 others did not receive any supplementation[31]. Background dietary vitamin D and calcium intakes were not assessed. 25(OH)D was measured only at the delivery (thus at the end of the supplementation period). At delivery, there was a pronounced difference in mean ± SEM 25(OH)D concentrations between women that received vitamin D supplementation (57 ± 11 nmol/L) and those that did not (25 ± 2 nmol/L) (t-test $p < 0.01$).

In the same open-label RCT, Ala-Houhala et al. (1986) also studied the effect of vitamin D supplementation in lactating women (49 starters, 49 completers)[32] (whose age was not reported) from January through March. Mothers received either no treatment (n = 16), 25 µg (n = 16) or 50 µg (n = 17) vitamin D_3 per day from delivery and until 15 weeks post partum. Background dietary vitamin D and calcium intakes were not assessed. At baseline, there were no significant differences in 25(OH)D concentrations across the three groups, showing mean concentrations around 32 nmol/L (concentration is estimated from figure in original article). However, after 15 weeks, 25(OH)D

[31] The study by Ala-Houhala et al. (1986) also included a third study group, including women that were supplemented during the second trimester of the pregnancy. As 25(OH)D measurements were only conducted at delivery, the data of this group that was supplemented in the second trimester were not considered relevant to this review (i.e. supplementation was terminated several months before the 25(OH)D measurements were conducted).

[32] Researchers already followed these lactating women during pregnancy, during which women were distributed over three groups: i.e. eight women were supplemented with 12.5 µg vitamin D_3 per day throughout the pregnancy; eight women were supplemented with 12.5 µg vitamin D_3 per day during the second trimester of pregnancy; 33 others did not receive any supplementation. After delivery, the women were re-distributed into three 'new' groups, as explained in the paragraph above.

concentration significantly increased in the treatment groups (paired t-tests P<0.01). That is, up to about 75 nmol/L in the 25 µg/day group and 100 nmol/L in the 50 µg/day group (concentrations are estimated from figure in original article).

The Panel considers that the two infants studies may be used to set DRVs for vitamin D in infants (Section 6.2.), while the other available studies on children, and pregnant or lactating women are not informative for the setting of DRVs for vitamin D for these population groups. The Panel also notes that ESPGHAN (Braegger et al., 2013) recommends the 'pragmatic use' of a serum 25(OH)D concentration of > 50 nmol/L to indicate sufficiency and a daily supplement of 10 µg to all infants. The Panel notes that mean vitamin D concentrations in breast milk of healthy lactating women, unsupplemented or supplemented with vitamin D, are low (0.25–2.0 µg/L) (Section 2.3.7.), that maternal vitamin D intake during lactation influence maternal serum 25(OH)D concentration, but is only modestly correlated with the amount of vitamin D in human milk, unless high supplemental doses are used. Thus, the Panel considers that the derivation of a DRV for infants in the second half of the first year of life by extrapolation from the vitamin D intake of breastfed infants is not possible, and that the compensation of the vitamin D loss in breast milk is not justified for the derivation of DRVs for vitamin D for lactating women.

6. Data on which to base Dietary Reference Values

In spite of the high variability in 25(OH)D measurements obtained with different analytical methods, the Panel nevertheless concludes that serum 25(OH)D concentration, which reflects the amount of vitamin D attained from both cutaneous synthesis and dietary sources, can be used as biomarker of vitamin D status in adult and children populations. Serum 25(OH)D concentration can also be used as biomarker of vitamin D intake in a population with low exposure to UV-B irradiation.

The Panel considers some musculoskeletal health outcomes as suitable to set DRVs for vitamin D for adults, infants and children (Sections 5.1.1 and 5.1.5). Taking into account the overall evidence and uncertainties on the relationship between serum 25(OH)D concentration and these health outcomes, the Panel concludes that a serum 25(OH)D concentration of **50 nmol/L is a suitable target value for all age and sex groups** (Section 5.1.5). For setting DRVs for vitamin D, the Panel considers the dietary intake of vitamin D necessary to achieve this serum 25(OH)D concentration. As for other nutrients, DRVs for vitamin D are set assuming that intakes of interacting nutrients, such as calcium (EFSA NDA Panel, 2015a), are adequate.

The Panel considers that the available evidence (Sections 5.1.5., 5.2.8. and 5.3.2.) does not allow the setting of ARs and PRIs) and chooses to **set AIs** instead, for all population groups.

6.1. Adults

The Panel used information obtained from characterising the intake-status relationship for vitamin D (Section 5.3.2) to derive the vitamin D intake to achieve a target serum 25(OH)D concentration of 50 nmol/L.

For the purpose of deriving AIs for vitamin D, the Panel decided to focus on the *adjusted* model of achieved mean serum 25(OH)D according to Ln (total vitamin D intake) (i.e. total intake from habitual diet, fortified foods or supplements). As indicated in Section 5.3.2., this adjusted model was obtained with data *mostly on adults* (74 arms out of 83 included arms) in randomised trials using *vitamin D_3* (not vitamin D_2) (Sections 2.3.2., 2.3.6., 5.3.2 and Appendix C), and the estimates from this adjusted model were derived based on all covariates set to their *mean* values.

In the *adjusted model*, the total intake estimated to achieve a serum 25(OH)D concentration of 50 nmol/L, as identified by the lower limit of the 95% PI, is *16.1* µg/day (Appendix C, Table 7). Equally, at a vitamin D intake of 15 µg/day, the predicted mean serum 25(OH)D concentration is 63 nmol/L (95% CI: 58–69 nmol/L), with a predicted value at the lower limit of the 95% PI of *49* nmol/L (Appendix C, Table 6).

The Panel notes that the PI in the context of a meta-regression analysis illustrates the uncertainty about the true *mean response* predicted in a future *study* (Section 5.3.). The Panel also considers that the 95% PI constitutes an *approximation* of the interval that would include 95% of all *individual responses* from the populations of interest, as it refers to the population of mean responses (Section 5.3.). The extent of this approximation could not be quantified.

The Panel therefore sets an AI for vitamin D for adults at 15 µg/day, considering that, at this intake, most of the adult population will achieve the target serum 25(OH)D concentration near or above 50 nmol/L. The Panel notes that this value for total intake of vitamin D is above the supplementation dose identified in Section 5.2.8. in relation to beneficial effect on musculoskeletal health outcomes. The Panel decided not to set specific AIs for 'younger' or 'older' adults, because there was no evidence of a significant difference in absorption capacity between 'younger' and 'older' adults (Section 2) and the majority of the studies used to set the target value for 25(OH)D concentration were carried out in 'older adults' (Section 5).

The *unadjusted* model (Appendix D.G) can be also taken into account as it encompasses the whole heterogeneity across trials. In the *unadjusted* model, considering a vitamin D intake of 15 µg/day, the *lower* limit of the 95% PI is *34* nmol/L. The Panel notes that this value of 34 nmol/L is above the concentrations that have been observed in relation to overt adverse health outcomes (Sections 5.1.1.1.2. and 5.1.1.1.6. on osteomalacia, calcium absorption). In addition, considering a vitamin D intake of 15 µg/day, the *upper* limit of the 95% PI is 91 nmol/L in the *unadjusted* model (and 78 nmol/L in the *adjusted* model). The Panel notes that these values are in the physiological range.

The Panel underlines that the meta-regression was done on data collected **under conditions of minimal cutaneous vitamin D synthesis**. In the presence of endogenous cutaneous vitamin D synthesis (Section 2.3.1), the requirement for dietary vitamin D is lower or may even be zero.

6.2. Infants

The Panel notes that there are few data on the relationship between 25(OH)D concentration and musculoskeletal health outcomes available in infants (Section 5.1.1.2.). The Panel notes that there are no data to suggest a different target value for 25(OH)D concentration for infants compared to the adult age group (Section 5.1.5.). The Panel also considers that, since breast milk does not supply adequate amounts of vitamin D to the breastfed infant (Section 2.3.7.2.), the derivation of an AI for infants in the second half of the first year of life by extrapolation from the vitamin D intake of breastfed infants is not possible (Section 5.3.3.).

In line with conclusions by the IOM (Section 4), the Panel notes that two recent trials (Holmlund-Suila et al., 2012; Gallo et al., 2013) (Sections 5.1.1.2 and 5.2.6.) showed that a supplementation with 10 µg/day vitamin D_3 in (mostly) breastfed infants was sufficient to reach a plasma/serum 25(OH)D of at least 50 nmol/L in (almost) all subjects. Only two studies (Ala-Houhala et al., 1986; Atas et al., 2013) that were conducted in breastfed infants in situation of low endogenous vitamin D synthesis met the eligibility criteria of the comprehensive literature search (Brouwer-Brolsma et al., 2016) mentioned previously (Section 5.3.3). Giving vitamin D supplementation of 10 µg/day to breastfed infants for at least 15 weeks led to an achieved serum 25(OH)D concentration of at least 80 nmol/L in both studies.

The Panel sets an AI for vitamin D for infants at 10 µg/day.

6.3. Children

The Panel notes that there are few data on the relationship between 25(OH)D concentration and musculoskeletal health outcomes available in children (Section 5.1.1.2.). The Panel notes that there are no data to suggest a different target value for 25(OH)D concentration for children compared to the adult age group (Section 5.1.5.).

The Panel sets an AI for vitamin D for adults at 15 µg/day, based on the analysis of the adjusted and unadjusted models of the meta-regression analysis (Sections 5.3.2. and 6.1. and Appendix C) that were obtained from data collected mostly on adults, but also on children. Thus, this value of 15 µg/day may also apply to children.

From Appendices C and D.G, a further stratified analysis by age group (adults versus children) (Section 5.3.2) showed that children tended to achieve the same mean serum 25(OH)D concentrations as the adults at a lower total intake (Appendix D.G). In addition, in the analysis based *only* on the four trials in children (age range: 2-17 years, nine arms), taking into account the limitations previously described in details (Section 5.3.2):

- In the *adjusted model* (adjusted only for baseline serum 25(OH)D concentration), the total intake estimated to achieve a serum 25(OH)D concentration of 50 nmol/L (Appendix D.G, Table 15), at the lower limit of the 95% CI, is 7.9 µg/day and at the lower limit of the 95% PI is *10.9* µg/day. In the *unadjusted* model, the total intake estimated to achieve a serum 25(OH)D concentration of 50 nmol/L, at the lower limit of the 95% CI, is *11.5* µg/day and, at the lower limit of the 95% PI, is 27.6 µg/day.

- Equally, at a vitamin D intake of 15 µg/day (Appendix D.G, Table 14), in the *adjusted model* (adjusted only for baseline serum 25(OH)D), the predicted mean serum 25(OH)D concentration is 67 nmol/L (95% CI: 61–73 nmol/L), with a predicted value at the lower limit of the 95% PI of *55* nmol/L. In the *unadjusted model*, at a vitamin D intake of 15 µg/day, the predicted mean serum 25(OH)D concentration is 73 nmol/L (95% CI: 56-91 nmol/L), with a predicted value at the lower limit of the 95% PI of *35* nmol/L. The Panel notes that this value of 35 nmol/L is above the concentrations that have been observed in relation to overt adverse health outcomes (Sections 5.1.1.2.2. on rickets).

The Panel sets an AI for vitamin D for all children (1–17 years) at 15 µg/day. The Panel underlines that the meta-regression was done on data collected **under conditions of minimal cutaneous vitamin D synthesis**. In the presence of endogenous cutaneous vitamin D synthesis (Section 2.3.1), the requirement for dietary vitamin D is lower or may even be zero.

6.4. Pregnancy

The Panel notes that there are no data to suggest a different target value for 25(OH)D concentration for pregnant women compared to non-pregnant women (Section 5.1.2.).

The Panel considers that the AI for pregnant women is the same as for non-pregnant women (15 µg/day). The Panel underlines that the meta-regression on adults (Sections 5.3 and 6.1) was done on data collected **under conditions of minimal cutaneous vitamin D synthesis**. In the presence of endogenous cutaneous vitamin D synthesis (Section 2.3.1), the requirement for dietary vitamin D is lower or may even be zero.

6.5. Lactation

The Panel notes that no studies were available for setting an AI for lactating women (Sections 5.1.3. and 5.3.3.). The Panel notes that mean vitamin D concentrations in breast milk of healthy lactating women, unsupplemented or supplemented with vitamin D, are low (0.25–2.0 µg/L), that maternal vitamin D intake during lactation influence maternal serum 25(OH)D concentration, but is only modestly correlated with the amount of vitamin D in human milk, unless high supplemental doses are used. The Panel considers that compensation of the vitamin D loss in breast milk is not justified for the derivation of DRVs for vitamin D for lactating women (Sections 2.3.7. and 5.3.3.).

The Panel considers that the AI for lactating women is the same as for non-lactating women (15 µg/day). The Panel underlines that the meta-regression on adults (Sections 5.3 and 6.1) was done on data collected **under conditions of minimal cutaneous vitamin D synthesis**. In the presence of endogenous cutaneous vitamin D synthesis (Section 2.3.1), the requirement for dietary vitamin D is lower or may even be zero.

CONCLUSIONS

The Panel concludes that ARs and PRIs for vitamin D cannot be derived for adults, infants and children, and therefore defines AIs, for all population groups. The Panel considers that serum 25(OH)D concentration, which reflects the amount of vitamin D attained from both cutaneous synthesis and dietary sources, can be used as biomarker of vitamin D intake in adult and children populations with low exposure to UV-B irradiation and as biomarker of vitamin D status. The Panel notes that the evidence on the relationship between serum 25(OH)D concentration and musculoskeletal health outcomes in adults, infants and children, and some adverse pregnancy-related health outcomes, is widely variable. Several factors contribute to this, and also include the large variation in the results from different laboratories and assays used for measuring serum 25(OH)D concentrations. Taking into account the overall evidence and uncertainties, the Panel considers that a serum 25(OH)D concentration of 50 nmol/L is a suitable target value for population groups, in view of setting the AIs for vitamin D.

For adults, the Panel sets an AI for vitamin D at 15 µg/day. This is based on the adjusted model of a meta-regression analysis of serum 25(OH)D concentration according to total vitamin D intake (natural log of the sum of habitual diet, and fortified foods or supplements using vitamin D_3). The Panel considers that, at this intake, most of the adult population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L. For children aged 1–17 years, the Panel sets an AI for vitamin D at 15 µg/day, based on the meta-regression analysis. For infants aged 7–11 months, the Panel sets an AI for vitamin D at 10 µg/day, based on four recent trials on the effect of vitamin D supplementation on serum 25(OH)D concentration in (mostly) breastfed infants. For pregnant and lactating women, the Panel considers that the AI is the same as for non-pregnant non-lactating women, i.e. 15 µg/day. The Panel underlines that the meta-regression in adults and children was done on data collected under conditions of minimal cutaneous vitamin D synthesis. In the presence of endogenous cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may even be zero.

Table 4: Summary of Dietary Reference Values for vitamin D

Age	AI [(a)] (µg/day)
7–11 months	10
1–3 years	15[(a)]
4–6 years	15[(a)]

7–10 years	15[(a)]
11–14 years	15[(a)]
15–17 years	15[(a)]
≥ 18 years [(b)]	15[(a)]

(a): **under conditions of minimal cutaneous vitamin D synthesis.** In the presence of endogenous cutaneous vitamin D synthesis (Section 2.3.1), the requirement for dietary vitamin D is lower or may be even zero.
(b): including pregnancy and lactation.

RECOMMENDATIONS FOR RESEARCH

Standardised investigations are needed to assess changes in musculoskeletal related health outcomes and surrogate markers in response to vitamin D_2 and D_3 intake, and in relation to serum 25(OH)D concentrations.

Studies specifically designed to identify cut-off values for 25(OH)D or other suitable biomarkers to derive DRVs for vitamin D for infants, children, adults, pregnant and lactating women.

The role of vitamin D status in non-musculoskeletal related health outcomes should be further explored.

More data on the effects of genotype/ethnicity and body fat mass on vitamin D metabolism and the requirements for vitamin D are warranted. More precise data on total vitamin D concentration in foods would also be useful.

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APPENDICES

Appendix A. Measurements for the assessment of bone health

Bone measurements in children and adults may be obtained using different techniques of bone densitometry, e.g. dual-energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT), peripheral quantitative computed tomography (pQCT) or quantitative ultrasound (QUS). Assessments of the advantages, precision, specificity and sensitivity of these methods in different populations (e.g. (Baroncelli, 2008; Brunner et al., 2011; Edelmann-Schafer et al., 2011) and recommendations on their use (e.g. from the International Society for Clinical Densitometry) have been published.

DXA is the most commonly used method of measuring bone mass. DXA measurements may include lumbar spine, hip, forearm and whole body. The DXA scans provide a number of outcomes: bone area, BMC and BMD in the above mentioned anatomical areas. BMD is a two-dimensional measurement of the bone, i.e. areal BMD (aBMD, $g \times cm^{-2}$). The calibration of the different DXA densitometers may differ between studies, resulting in different BMD and BMC values.

In contrast, QCT, which also involves x-ray radiation, is used to measure three-dimensional (volumetric) BMD ($g \times cm^{-3}$) in the spine or hip, and to assess bone structure, i.e. separately analyse BMD for the compact (or cortical) bone or for the trabecular (or cancellous) bone. Moreover, pQCT measures bone characteristics in 'peripheral' body sites such as the forearms or legs and provides a number of outcomes, e.g. volumetric BMD (vBMD), the stress-strain index (SSI) and measures of the geometry of the bone (i.e. spatial distribution of the bone mass) (Section 5.1.1.2.). QUS methods have been developed to give estimates of bone health, without the use of ionising radiation. Measurements are usually performed at the heel (calcaneus). In its review, the Panel did not identify any recent relevant study on bone-related outcomes using this technique.

Appendix B. Summary of the evidence considered by the IOM to set DRVs for vitamin D

1. Adults

IOM (2011) used mostly the systematic reviews by Cranney et al. (2007) and by Chung et al. (2009) to draw conclusions on 25(OH)D concentrations and bone-related health outcomes.

Cranney et al. (2007) considered nineteen studies on the association between **serum 25(OH)D concentrations and BMD in older adults**. They comprised six RCTs on vitamin D supplementation with calcium (Dawson-Hughes et al., 1995; Storm et al., 1998; Schaafsma et al., 2002; Cooper et al., 2003; Aloia et al., 2005) or without calcium (Ooms et al., 1995). These RCTs and two cohort studies (Dennison et al., 1999; Gerdhem et al., 2005) reported no significant association between serum 25(OH)D concentrations and BMD or bone loss. However, five other cohort studies reported a significant association, particularly at the hip sites (Rosen et al., 1994; Stone et al., 1998; Melin et al., 2001; del Puente et al., 2002; Bischoff-Ferrari et al., 2005), and only one at the lumbar spine (Rosen et al., 1994). Six case-control studies (Villareal et al., 1991; Thiebaud et al., 1997; Boonen et al., 1999; Landin-Wilhelmsen et al., 1999; Yan et al., 2003; Al-oanzi et al., 2006) reported an association between 25(OH)D concentrations and BMD, most consistently at the femoral neck. Chung et al. (2009) included two additional RCTs (Andersen et al., 2008; Zhu et al., 2008b). Zhu et al. (2008b) showed that vitamin D_2 supplementation over one year provided no extra benefit in older Caucasian women (mean baseline serum 25(OH)D concentration: 44.3 nmol/L) on total hip BMD compared to calcium supplementation alone. Andersen et al. (2008) reported no effect of the vitamin D_3 supplementation on BMC/BMD and no differences in one-year BMD changes at the lumbar spine between the intervention and placebo groups, either in female or in male Pakistani immigrants in Denmark (mean baseline serum 25(OH)D concentration: 12 (women) and 21 (men) nmol/L).

With regards to **vitamin D supplementation with or without calcium in older adults and BMD**, Cranney et al. (2007) identified 17 RCTs (Dawson-Hughes et al., 1991; Chapuy et al., 1992; Dawson-Hughes et al., 1995; Ooms et al., 1995; Dawson-Hughes et al., 1997; Baeksgaard et al., 1998; Komulainen et al., 1998; Hunter et al., 2000; Patel et al., 2001; Chapuy et al., 2002; Jensen et al., 2002; Cooper et al., 2003; Grados et al., 2003; Harwood et al., 2004; Meier et al., 2004; Aloia et al., 2005; Jackson et al., 2006), mostly in post-menopausal women and older men (i.e. (Patel et al., 2001; Meier et al., 2004) also included younger subjects). Combining results of individual studies to calculate weighted mean differences, Cranney et al. (2007) concluded that vitamin D_3 plus calcium supplementation compared with placebo resulted in 'small' significant increases in BMD of the lumbar spine, total body and femoral neck (but not of the forearm). However, they concluded that vitamin D_3 plus calcium compared with calcium did not have a significant effect on BMD of the lumbar spine, total hip, forearm or total body (but the effect for femoral neck was significant). They also concluded that vitamin D_3 supplementation alone versus placebo had a significant effect on BMD at the femoral neck but not at the forearm. Chung et al. (2009) identified three additional RCTs in older adults (Moschonis and Manios, 2006; Bolton-Smith et al., 2007; Zhu et al., 2008a), only two of which (Moschonis and Manios, 2006; Zhu et al., 2008a) found a significant increase in hip or total BMD in postmenopausal women receiving vitamin D_2 or D_3 plus calcium compared with placebo.

For **osteomalacia**, the IOM used a study on *post-mortem* biopsies (Priemel et al., 2010) (Section 5.1.1.1.2).

For **fracture risk in older adults**, **with regard to serum 25(OH)D concentrations**, Cranney et al. (2007) identified only observational studies. They took into account three prospective cohort studies in independently living older adults (Woo et al., 1990; Cummings et al., 1998; Gerdhem et al., 2005). They also considered case-control studies (Lund et al., 1975; Lips et al., 1983; Punnonen et al., 1986; Lips et al., 1987; Cooper et al., 1989; Lau et al., 1989; Boonen et al., 1997; Thiebaud et al., 1997; Diamond et al., 1998; Boonen et al., 1999; Landin-Wilhelmsen et al., 1999; LeBoff et al., 1999; Erem et al., 2002; Bakhtiyarova et al., 2006). Cranney et al. (2007) concluded that there was inconsistent

evidence for an association between a lower serum 25(OH)D concentration and an increased risk of fracture. IOM (2011) identified six additional observational studies (Cauley et al., 2008; Looker and Mussolino, 2008; van Schoor et al., 2008; Ensrud et al., 2009; Cauley et al., 2010; Melhus et al., 2010). These showed inconsistent results on 25(OH)D concentrations below which there may be an increased risk of fracture, which varied between 30 to 70 nmol/L.

With regard to vitamin D supplementation and risk of fractures, Cranney et al. (2007) assessed 15 RCTs (Chapuy et al., 1992; Lips et al., 1996; Dawson-Hughes et al., 1997; Komulainen et al., 1998; Pfeifer et al., 2000; Chapuy et al., 2002; Trivedi et al., 2003; Anderson et al., 2004; Harwood et al., 2004; Larsen et al., 2004; Flicker et al., 2005; Grant et al., 2005; Porthouse et al., 2005; Jackson et al., 2006; Law et al., 2006). These RCTs investigated the effect of vitamin D (with or without calcium) on fractures in postmenopausal women and older men with baseline 25(OH)D concentrations ranging from 22 to 82.7 nmol/L. Eleven of these RCTs used vitamin D_3 preparations (7.5–20 µg/day), and the others vitamin D_2 (Anderson et al., 2004; Larsen et al., 2004; Flicker et al., 2005; Law et al., 2006). Cranney et al. (2007) conducted a meta-analysis of 13 of these RCTs, omitting the abstract by Anderson et al. (2004) and the study by Larsen et al. (2004) with no placebo control. Cranney et al. (2007) calculated combined ORs that indicated non-significant effect of the interventions for total fractures,[33] non-vertebral fractures,[34] hip fractures,[35] vertebral fractures,[36] and total or hip fractures in community-dwelling older adults. Combined ORs also indicated significant reduction in the risk of fractures for end of study 25(OH)D concentration ≥ 74 nmol/L (compared to 25(OH)D < 74 nmol/L),[37] and for total or hip fractures in institutionalised older adults.[38] Chung et al. (2009) identified three additional RCTs on bone health (Bunout et al., 2006; Burleigh et al., 2007; Lyons et al., 2007), two of which investigated fracture risk. These did not show significant effects of either vitamin D_2 (four-monthly dose equivalent to 20.6 µg/day) compared with placebo, or of vitamin D_3 (20 µg/day) plus calcium compared with calcium, in reducing the risk of total fractures, in a cohort of hospital inpatients (Burleigh et al., 2007) and in older adults living in residential or care homes (Lyons et al., 2007). IOM (2011) identified two additional RCTs (Salovaara et al., 2010; Sanders et al., 2010). In both studies, there was no statistically significant effect of the combination of calcium and vitamin D_3 on incident fractures compared to no treatment.

Based on Cranney et al. (2007) and Chung et al. (2009) and observational data outside of these reviews (four other cross-sectional (Bischoff-Ferrari et al., 2004; Boxer et al., 2008; Stewart, 2009) or longitudinal (Wicherts et al., 2007) observational studies), IOM (2011) found that there was some support for an association between 25(OH)D concentrations and **physical performance** (data for this outcome were considered together with that for the risk of falls mentioned below). However, IOM (2011) found that high-quality and large observational cohort studies were lacking, and that randomised trials suggest that vitamin D dosages of at least 20 µg/day, with or without calcium, may improve physical performance measures. Although high doses of vitamin D (i.e., ≥ 20 µg/day) may provide greater benefit for physical performance than low doses (i.e., 10 µg/day), the IOM found that the evidence was insufficient to define the shape of the dose–response curve for higher levels of intake.

Based on Cranney et al. (2007) and Chung et al. (2009) and two RCTs (Bischoff-Ferrari et al., 2010; Sanders et al., 2010) published afterwards, IOM (2011) considered that no consistent outcome was

[33] Vitamin D_2 or D_3 +/- calcium compared with calcium or placebo, vitamin D_3 compared with placebo, vitamin D_3 + calcium compared with calcium.
[34] Vitamin D_3 compared with placebo, vitamin D_3 + calcium compared placebo.
[35] Vitamin D_3 compared with placebo, vitamin D_3 + calcium compared with calcium, vitamin D_3 + calcium compared placebo.
[36] Vitamin D_2 or D_3 +/- calcium compared with calcium or placebo.
[37] In four trials using vitamin D_3 with end of study 25(OH)D concentrations of >74 nmol/L, out of 10 trials reporting follow-up or change in mean 25(OH)D concentrations.
[38] Older adults receiving vitamin D_2 or D_3 with calcium, compared to calcium or placebo (three trials on total fractures), or vitamin D_3 with calcium, compared to placebo (two trials on hip fractures, combined OR: 0.69; 95 % CI: 0.53–0.90).

found from randomised trials that tested for effects of vitamin D with and without calcium on reduction in **risk for falls**. IOM considered 20 randomised trials on oral doses (Graafmans et al., 1996; Pfeifer et al., 2000; Chapuy et al., 2002; Bischoff et al., 2003; Trivedi et al., 2003; Flicker et al., 2005; Grant et al., 2005; Larsen et al., 2005; Bischoff-Ferrari et al., 2006; Law et al., 2006; Broe et al., 2007; Burleigh et al., 2007; Prince et al., 2008; Pfeifer et al., 2009; Bischoff-Ferrari et al., 2010) or injected doses (Latham et al., 2003; Dhesi et al., 2004; Harwood et al., 2004; Smith et al., 2007; Sanders et al., 2010). These RCTs had heterogeneous designs, e.g. subjects were either free-living or institutionalised older subjects, and supplemented with vitamin D with or without calcium and compared to calcium or placebo. From these, IOM noted that only four (Pfeifer et al., 2000; Harwood et al., 2004; Flicker et al., 2005; Broe et al., 2007) found a significant effect of vitamin D on fall incidence, and that the only two significant studies for fallers were Pfeifer et al. (2000); Pfeifer et al. (2009).[39] The IOM (2011) noted that a number of the RCTs analysed falls rather than fallers. The IOM concluded that the greater part of the causal evidence indicated no significant reduction in fall risk related to vitamin D intake or achieved concentration in blood. IOM (2011) noted that Cranney et al. (2007)[40] and Chung et al. (2009) found no consistency between study findings. With regard to the evidence from observational studies, the IOM noted one longitudinal Dutch study (Snijder et al., 2006) (which was not part of Cranney et al. (2007) or Chung et al. (2009)) that found that a serum 25(OH)D concentration < 25 nmol/L was independently associated with an increased risk of falling for subjects who experienced two or more falls compared with those who did not fall or fell once. IOM (2011) summarised that observational studies suggested an association between a higher serum 25(OH)D concentration and a lower risk of falls in older adults.

In relation to **calcium absorption in adults**, IOM (2011) considered RCTs in mainly postmenopausal women with vitamin D supplementation (Francis et al., 1996; Patel et al., 2001; Zhu et al., 2008b; Zhu et al., 2008a), using the dual isotope technique. The RCTs varied considerably in design and, overall, showed no effect of increasing the serum 25(OH)D concentrations on intestinal calcium absorption compared with placebo. In a short-term RCT in postmenopausal women using dual isotope technique, Hansen et al. (2008) showed a 3% increase in absorption after raising the serum 25(OH)D concentration from 55 to 160 nmol/L. IOM also considered cross-sectional studies using the single-isotope technique (Kinyamu et al., 1998; Devine et al., 2002; Heaney et al., 2003b; Need et al., 2008; Aloia et al., 2010). In particular, in 319 patients (mostly men) attending osteoporosis clinics and with serum 25(OH)D concentrations less than 40 nmol/L, Need et al. (2008) found no increase in fractional calcium absorption in subjects with serum 25(OH)D concentrations above 10 nmol/L. The studies by Heaney et al. (2003b) and Kinyamu et al. (1998) indicated no changes in fractional calcium absorption across ranges of serum 25(OH)D concentrations of 60–154 nmol/L and 50–116 nmol/L, respectively. In the study by Aloia et al. (2010) in 492 African American and 262 Caucasian women (20–80 years), no relationship was found between calcium absorption and serum 25(OH)D concentrations ranging from 30 to 150 nmol/L. The relationship between calcium absorption and 1,25$(OH)_2$D concentration was positive and stronger for lower than for higher 25(OH)D concentrations.

IOM (2011) concluded that serum 25(OH)D concentrations of **40 nmol/L, 50 nmol L or higher** were sufficient to meet **bone health** requirements for most **adults** in RCTs, and to provide maximal population coverage in observational studies on adults and bone health.

[39] In a sensitivity analysis, Cranney et al. (2007) found that combining the results from eight trials on oral vitamin D_2 or D_3 with calcium, compared to placebo or calcium alone, showed a significant reduction in the risk of falls (OR: 0.84 ; 95% CI: 0.76–0.93), heterogeneity $I^2 = 0\%$).

[40] In total, Cranney et al. (2007) identified one RCT, three cohorts and one case-control on the association between serum 25(OH)D concentrations and risk of falls, as well as three RCTs and four cohorts on the association between 25(OH)D concentrations and measures of performance (among these, one cohort investigated both risk of falls and measures of performance). Chung et al. (2009) identified three additional RCTs on vitamin D supplementation and the risk of falls, including one which also investigated measures of performance, and one additional RCTs on vitamin D with calcium and measures of performance.

2. Infants and children

For infants, Cranney et al. (2007) reported on the inconsistent results of two RCTs with vitamin D_2 supplementation examining serum 25(OH)D concentrations and **BMC** (Greer et al., 1982; Greer and Marshall, 1989), and on the inconsistent results of three case-control studies (Bougle et al., 1998; Namgung et al., 1998; Park et al., 1998) examining serum 25(OH)D concentrations and **BMD and/or BMC**. Chung et al. (2009) found no additional RCTs in infants.

For children, Cranney et al. (2007) identified three RCTs (Ala-Houhala et al., 1988b; El-Hajj Fuleihan et al., 2006; Viljakainen et al., 2006b), two prospective cohort studies (Lehtonen-Veromaa et al., 2002; Javaid et al., 2006), and one case–control study (Marwaha et al., 2005). In children (8-10 years) receiving vitamin D_2 supplementation or placebo for more than one year (Ala-Houhala et al., 1988b), the change in serum 25(OH)D concentrations after supplementation was not accompanied by a change in distal radial BMC. However, Cranney et al. (2007) reported, in girls (10–17 years) receiving two doses of vitamin D_3 supplementation or a placebo for one year (El-Hajj Fuleihan et al., 2006), that baseline serum 25(OH)D concentrations were significantly related to baseline BMD (positively) or percent change in BMC (negatively), at the lumbar spine, femoral neck, and radius. They also reported a significant increase in BMC only of the total hip in girls receiving the highest dose of supplementation, compared with placebo (El-Hajj Fuleihan et al., 2006). In girls (11-12 years) with 'adequate' calcium intake and who received one of two doses of daily vitamin D_3 supplementation or a placebo for one year, mean achieved serum 25(OH)D was above 50 nmol/L in both intervention groups (Viljakainen et al., 2006b). A significant increase in BMC of the femur (for both doses) or lumbar spine (for the highest dose) was reported in subjects with compliance above 80 %, but this was not statistically significant in the ITT analysis. Cranney et al. (2007) reported a positive association between baseline serum 25(OH)D concentrations of girls (9-15 years) followed for three years and change in BMD (Lehtonen-Veromaa et al., 2002), and between maternal serum 25(OH)D during pregnancy and BMC of the children (8–9 years) (Javaid et al., 2006). However, there was no significant correlation between serum 25(OH)D and BMD of children (10–18 years) in either group of the case-control study (Marwaha et al., 2005).

Cranney et al. (2007) concluded that there was evidence of an association between serum 25(OH)D concentrations and baseline BMD and change in BMD or related variables, but that the results of RCTs were not consistent with regard to the effect of vitamin D supplementation on BMD or BMC across skeletal sites and age groups. Chung et al. (2009) identified one RCT in 26 healthy Pakistani immigrant girls (10–17 years) living near Copenhagen (mean baseline 25(OH)D concentration: 11 nmol/L), and receiving one of two doses of vitamin D_3 supplementation alone or a placebo (Andersen et al., 2008). There were no significant differences in whole-body BMC changes between the supplemented groups and the placebo group. Chung et al. (2009) identified another RCT (Cheng et al., 2005) in healthy girls (10–12 years) (mean baseline 25(OH)D concentration: 35 nmol/L) receiving supplementation with vitamin D_3 and calcium or a placebo, which showed no significant difference in BMC changes between groups after two years.

According to IOM (2011) and Cranney et al. (2007), among 13 studies on **rickets,** six (including one RCT (Cesur et al., 2003)) reported mean or median serum 25(OH)D concentrations below 27.5 nmol/L, and expressed as about 30 nmol/L, in children with rickets (Garabedian et al., 1983; Markestad et al., 1984; Bhimma et al., 1995; Majid Molla et al., 2000; Cesur et al., 2003; Dawodu et al., 2005). The others (before-after or case-control) studies were reported as showing mean/median serum 25(OH)D concentrations higher than 30 nmol/L and up to 50 nmol/L in children with rickets (Arnaud et al., 1976; Elzouki et al., 1989; Oginni et al., 1996; Thacher and 1997; Thacher et al., 2000; Balasubramanian et al., 2003; Graff et al., 2004). Seven case–control studies showed lower serum 25(OH)D concentrations in cases than in controls (Arnaud et al., 1976; Oginni et al., 1996; Majid Molla et al., 2000; Thacher et al., 2000; Balasubramanian et al., 2003; Graff et al., 2004; Dawodu et al., 2005). Three studies were conducted in Western countries (Arnaud et al., 1976; Garabedian et al., 1983; Markestad et al., 1984), while most were conducted in non-Western countries with low calcium

intake. Cranney et al. (2007) noted that low calcium intake can influence the relationship between serum 25(OH)D and rickets and that the 25(OH)D cut-off value for rickets in populations with high calcium intake is unclear. Chung et al. (2009) did not identify any additional study on rickets.

For children, IOM (2011) identified two dual-isotope studies (an observational study (Abrams et al., 2009) or a randomized trial (Thacher et al., 2009)) on **fractional calcium absorption**, and a pooled analysis of several three-week calcium-balance metabolic studies in 105 girls (11–15 years) (Weaver et al., 2008), in which serum 25(OH)D concentration was not related to net calcium absorption or retention. However, in this last study, calcium balance or retention was calculated by subtracting calcium excretion through urine and faeces from dietary calcium intake. Pooling studies in 251 children (about 5–17 years) and assessing the relationship of 25(OH)D concentration (as a continuous variable) with either fractional or total calcium absorption, according to pubertal status and/or calcium intake, Abrams et al. (2009) found inconsistent results. However, when 25(OH)D was studied as a categorical variable in the whole population, fractional calcium absorption adjusted (in particular) for calcium intake was slightly, but significantly ($p < 0.05$), higher at 25(OH)D concentration of 28–50 nmol/L, compared with ranges of 50–80 nmol/L or greater than 80 nmol/L. In Nigeria, 17 prepubertal children, with rickets, 'low' calcium intake and mean baseline 25(OH)D concentration of 50 nmol/L, were randomised to receive single oral supplementation of vitamin D_2 or D_3 (Thacher et al., 2009). An increase in serum 25(OH)D concentrations was reported in both groups, but at "low" calcium intake and with no significant increase in fractional calcium absorption between baseline and three days after supplementation (Thacher et al., 2009).

3. Pregnancy

For IOM (2011), during pregnancy, **maternal** $1,25(OH)_2D$ increases, while 25(OH)D is generally unaffected in unsupplemented women. Animal data reviewed by IOM (2011) suggested that the increased **calcium absorption** during pregnancy is independent from vitamin D or $1,25(OH)_2D$, and observational data showed that vitamin D-deficiency **rickets** may develop weeks or months after birth. For **maternal bone health** during pregnancy, Cranney et al. (2007) identified two prospective observational studies (Ardawi et al., 1997; Morley et al., 2006) and one before-and-after study (Datta et al., 2002), which found either a negative or no correlation between maternal serum 25(OH)D and PTH concentrations. Maternal BMD/BMC was not investigated in these studies. Chung et al. (2009) or IOM (2011) identified no RCTs for this outcome.

For the prevention of **pre-eclampsia**, the IOM noted the absence of placebo-controlled RCTs in favour of an effect of vitamin D. One RCT (Marya et al., 1987) (identified by Chung et al. (2009)) found no effect of vitamin D and calcium supplementation on the incidence of pre-eclampsia and the results of a non-randomised trial on vitamin D_3 and calcium supplementation (Ito et al., 1994) were found unclear. Two observational studies showed inverse associations between vitamin D intake from supplements and risk of pre-eclampsia (Hypponen et al., 2007; Haugen et al., 2009). For the IOM, case-control or nested case-control studies (including one (Bodnar et al., 2007) found by Chung et al. (2009)), investigating serum 25(OH)D concentration and the risk of pre-eclampsia or comparing serum 25(OH)D concentration in women with or without pre-eclampsia, found contradictory results (Frolich et al., 1992; Seely et al., 1992; Bodnar et al., 2007). However, one case-control study (Lalau et al., 1993) showed lower total or free serum $1,25(OH)_2D$ in women with pregnancy-induced hypertension.

The IOM noted the limited observational evidence on **non-skeletal maternal outcomes** (caesarean section, obstructed labour, vaginosis), reviewed neither in Cranney et al. (2007) nor in Chung et al. (2009). In RCTs (most identified by Chung et al. (2009)) on maternal vitamin D supplementation and **birth weight or length** (Brooke et al., 1980; Maxwell et al., 1981; Mallet et al., 1986; Marya et al., 1988), no effect was observed. IOM also reported on observational studies with conflicting results on vitamin D intake/status during pregnancy and **infant birth size or small-for-gestational age**

measurements (Brunvand et al., 1998; Morley et al., 2006; Gale et al., 2008; Farrant et al., 2009; Scholl and Chen, 2009; Bodnar et al., 2010; Leffelaar et al., 2010).

For fetal/newborn bone health, an RCT (Delvin et al., 1986) was reported as showing no effect of maternal vitamin D supplementation on fetal calcium homeostasis. The IOM also considered observational studies (Maxwell and Miles, 1925; Brooke et al., 1980; Congdon et al., 1983; Silver et al., 1985; Pereira and Zucker, 1986; Campbell and Fleischman, 1988; Specker et al., 1992; Specker, 1994; Takeda et al., 1997; Teotia and Teotia, 1997; Kitanaka et al., 1998; Akcakus et al., 2006; Bouillon et al., 2006; Beck-Nielsen et al., 2009). From them, the IOM concluded that there was no relationship between maternal 25(OH)D concentration and fetal BMC or BMD, as well as normal fetal skeletal development and no radiological evidence of rickets at birth in case of maternal vitamin D 'deficiency' or absence of 1α-hydroxylase or the VDR. Other observational studies were reported as showing lower maternal and neonatal serum 25(OH)D concentrations in infants with craniotabes (Reif et al., 1988) and an inverse association between fetal femur metaphyseal cross–sectional area or splaying index and maternal 25(OH)D during pregnancy (Mahon et al., 2010). From another observational study (Viljakainen et al., 2010), the IOM noted the lower newborn tibia BMC and cross–sectional area with maternal serum 25(OH)D concentration below 42.6 nmol/L (mean of first trimester and two-day post-partum values, close to the 'EAR-type value' proposed by the IOM), compared to higher serum 25(OH)D, after adjustments for potential confounders.

Regarding the relationship between maternal 25(OH)D during pregnancy and **childhood bone health**, the IOM refers to a study providing follow-up data on 33 % of the children included in a mother-infant cohort (n = 596 initially) (Javaid et al., 2006). This observational study reported a positive association between whole-body and lumbar spine BMC and aBMD in children (nine years) and maternal serum 25(OH)D concentrations in pregnancy (mean: 34 weeks) after adjustments for potential confounders. Children of mothers whose serum 25(OH)D concentrations in pregnancy were less than 27.5 nmol/L (compared to above 50 nmol/L) had a significantly lower whole-body BMC ($p = 0.002$).

4. Lactation

IOM (2011) stated that breast milk is not a significant source of vitamin D for breastfed infants, and that the maternal skeleton recovers BMC after the end of lactation. IOM (2011) considered observational studies (Cancela et al., 1986; Okonofua et al., 1987; Kent et al., 1990; Alfaham et al., 1995; Cross et al., 1995; Sowers et al., 1998; Ghannam et al., 1999) and intervention studies (Greer et al., 1982; Rothberg et al., 1982; Ala-Houhala, 1985; Ala-Houhala et al., 1988b; Greer and Marshall, 1989; Takeuchi et al., 1989; Kalkwarf et al., 1996; Hollis and Wagner, 2004b; Basile et al., 2006; Wagner et al., 2006; Saadi et al., 2007). Some of these had been identified by Cranney et al. (2007) and Chung et al. (2009). From these studies, the IOM reported no major change in serum 25(OH)D concentration during lactation compared to non-lactating women, and that providing vitamin D to lactating mothers increased their serum 25(OH)D concentrations, without significant effect on either infant serum 25(OH)D concentrations (for supplementation below 100 µg/day) or infant weight or height. The IOM also noted the lack of association between maternal 25(OH)D concentration and maternal post partum changes in BMD (e.g. lumbar spine or femoral neck), or breast milk calcium content (Prentice et al., 1997). IOM (2011) noticed that no RCTs had investigated the influence of maternal vitamin D intake or status on the recovery of maternal skeletal mineral content after the end of lactation.

Appendix C. Dose-response analysis undertaken by EFSA of serum 25(OH)D to total vitamin D intake: methods and key results

The specific objective of the quantitative analysis was to estimate the dose-response relationship between vitamin D total intake and plasma/serum 25(OH)D concentration in situations of assumed minimal endogenous vitamin D synthesis through exposure to the sun or artificial ultraviolet (UV) radiation in the healthy population.

The analysis as detailed in Appendix J was developed based on the related Analysis Plan, which has been informed by the systematic review protocol drafted by the contractor (Brouwer-Brolsma et al., 2016) in agreement with EFSA and by specific input from the NDA WG on Dietary Reference Values for Vitamins.

Data synthesis: meta-analyses, meta-regression, dose-response models

1. Criteria under which study data were quantitatively synthesised

In a meta-analytic approach, quantitative synthesis is usually carried out if included studies are sufficiently homogeneous to allow for meaningful combined estimates.

In the context of the current analysis a high statistical heterogeneity across included studies was expected; the relative contributions of methodological heterogeneity and/or 'clinical' heterogeneity were evaluated by analysing the relevant data extracted at the study level (e.g. dimensions of methodological quality, intake-status influencing factors).

In recognition of such heterogeneity, prospective observational studies were analysed separately from randomised trials, the latter being the basis for the dose-response modelling.

Once the methodological heterogeneity possibly due to differences in the internal validity of the results from individual studies is characterised, the remaining variation is likely to reflect a real phenomenon that describes the extent to which different populations behave differently. Independently of the extent to which identified 'clinical' covariates could explain it, heterogeneity was incorporated in the derivation of DRVs, in the idea that they are being applied to different populations in different contexts.

The very high heterogeneity was taken into account in meta-analyses and meta-regressions applying a random-effects model. **A random-effects model assumes that true effects follow a normal distribution around a pooled weighted mean (or around the conditional linear predictor for models) and allows for the residual heterogeneity among responses not characterised by subgroups analyses (or not modelled by the explanatory variables included in the multivariable models).**

All statistical analyses were performed with STATA version 13.1 (Stata-Corp, College Station, TX, USA). Unless otherwise specified, all estimates were presented with 95% confidence intervals (Cis) and all analyses were carried out at the level of statistical significance of 0.05.

2. Summary measures

The continuous outcome (i.e. plasma/serum 25(OH)D as a marker of vitamin D status) was analysed using the summary data extracted by the contractor (Brouwer-Brolsma et al., 2016) for each arm in each individual study: the number of participants included (and assessed); the mean values and SDs of the baseline and final values of 25(OH)D (as reported in the original paper or as converted by the contractor to nmol/L) at each relevant time point (i.e. final concentrations measured in a period of

assumed minimal endogenous vitamin D synthesis) and for each vitamin D dose/intake (up to 50 µg/day dose).

Summary measures and related standard errors were either calculated or imputed based on the type of summary data available (e.g. means were estimated from medians when these were available). Absolute achieved means and their standard errors were meta-analysed and used in the dose-response meta-regression models. Weighted mean differences (with 95% CI) as calculated by pooling study-specific estimates (when a control arm was available) in random-effects meta-analyses were used for comparative purposes. Net changes from baseline to achieved means by arm were calculated to check for consistency of results and to identify heterogeneity potentially due to methodological issues.

3. Unit of analysis issues

All included trials were assessed in order to check whether the unit of randomization was consistent with the unit of analysis in the trial (i.e. per individual randomised).

Only one cross-over trial was initially included (Patel et al., 2001), which was treated according to the contractor's criteria (i.e. only the two periods from November through February were considered eligible and extracted as two different studies: Patel et al., 2001a and Patelet al., 2001b). The trial was subsequently excluded based on its design and net change values (Appendix D.A).

4. Dealing with missing data

The contractor contacted the original authors of the individual studies to obtain relevant missing data; imputation was used in the current analysis (e.g. mean age derived from age range) to deal with key summary information that could not be retrieved despite the contractor's efforts.

Specific formulae (Higgins et al., 2011) were applied to derive summary data where not directly extracted/available in the format of the statistics mentioned in section 1.3 (e.g. SDs were calculated from standard errors and group size or from CIs). If no calculation/estimation was possible, the missing data were imputed according to the approach proposed by Wan et al. (2014).

Information for all relevant study-level characteristics was complete with the exceptions of funding source (6% missing), ethnicity (47%) and mean Body Mass Index (28%) (Appendix D.B, Table 9, Table 10 and Table 11). Availability of BMI mean values in the final dataset was maximised by calculating it from mean weight and mean height (BMI = body weight (kg) / height^2 (meters)) when available; missing data proportion dropped to 16%. While developing the final model, BMI missing data were included in a specific category as 'not reported', to be able to compare models with and without BMI as covariate (i.e. assuring same number of arms in all models). Funding and ethnicity were analysed likewise, although the high proportion of missing values for ethnicity prevented it from being included in the final model.

Background intake estimates were added to the supplemental vitamin D dose to generate total vitamin D intake estimates. If the habitual vitamin D intake of the cohort(s) within a study was not reported, surrogates were imputed using the appropriate age- and sex- specific mean vitamin D intake values (from food) from the national nutrition survey relevant to the country in which the study was performed (17 studies - Appendix D.B, Table 11); values were weighted for the arm-specific sex proportions and age ranges.

Only for one trial (Rich-Edwards et al., 2011) on children from Mongolia values were imputed from another included trial (Madsen et al., 2013)) on children from Denmark, as participants were of comparable age.

Sensitivity analyses to assess the impact of summary data and background intake imputations on the overall analyses were performed; the intake coefficient estimated in the dose-response model with no covariates on the revised data did not change substantially from the intake coefficient on the original values, showing an overall minor impact of imputation on the crude dose-response relationship.

5. Assessment of heterogeneity

Statistical heterogeneity was tested using the χ^2 test (Cochran's Q test; significance level: 0.10) and quantified by calculating the I^2 statistic (Higgins and Thompson, 2002).

I^2 ranges between 0 and 100 per cent and quantifies the proportion of the variability in effect estimates that can be attributed to heterogeneity rather than chance. As a reference, 0% to 40% might not be important; 30% to 60% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity; 75% to 100% represents considerable heterogeneity (Higgins et al., 2011).

I^2 was 99% in the overall meta-analysis of achieved mean values and did not drop below 94% in any sub-groups except when intervention doses were investigated (85% in trials with dose = 20 µg/day, 76% in trials with dose = 50 µg/day). Given the very high level of heterogeneity between trials possible sources were explored by subgroup analysis, meta-regression and/or sensitivity analysis.

6. Data checking

For each variable, the proportion of missing observations was calculated and range checks carried out to ensure that all values were plausible. The distributions of continuous variables were explored graphically and the frequency distributions of categorical variables tabulated. Key variables were cross-tabulated or scattered against each other to check for consistency. Summary data were double checked against original publications whenever deemed necessary and unit conversions of all included 25(OH)D and vitamin D dose/intake values were verified (ng/mL converted to nmol/L by multiplying by 2.496; IU/day converted to µg/day by dividing by 40).

7. Meta-analyses

Random-effects meta-analyses of summary response measures were carried out using the DerSimonian and Laird approach (DerSimonian and Laird, 1986), which encompasses both variability due to chance (i.e. the within-study variance component in the denominator of the individual study weight) and variability due to heterogeneity (i.e. the between-study variance component added in the denominator of the individual study weight - T^2 statistic).

Studies included in the meta-analyses

The mean responses measured as achieved 25(OH)D serum concentration in trial arms (both placebo/control and intervention groups) in a period of assumed minimal endogenous vitamin D synthesis were included in the preliminary analyses as long as the related individual trial arms met the following inclusion criteria:

- Young and older adults as well as children – no pregnant, no lactating, no infants (following discussion with WG members, as these represent particular age/physiological conditions),
- Vitamin D_3 only (as discussion with WG members suggested that intake of vitamin D_2 may have a different impact on 25(OH)D concentration),
- Summary data available or possible to estimate/impute,

- Dose of supplemented vitamin D ≤ 100 µg/day (Tolerable Upper Intake Level set by EFSA for adults (EFSA NDA Panel, 2012a)).

The inclusion criteria were applied at the arm level, as individual arms were considered the unit of analysis (except when mean differences were analysed).

After applying the inclusion criteria 116 arms (49 trials) out of the 141 available in the contractor's data set (57 trials from 49 articles[41]) were left for the preliminary analyses (Appendix D.A, Table 8, third column).

Upon evaluation of inconsistencies and outliers a further 33 arms were excluded from the preliminary data set (Appendix D.A, Table 8 - fourth column); the final data set included 83 arms from 35 trials (Appendix D.B), of which four studies (nine arms) were carried out on children (overall age range: 2-17 years).

Absolute achieved mean values and mean differences were analysed to check for the inclusion of trials/arms in the dose-response analysis (preliminary meta-analyses) and to complement the results from the dose-response models (final meta-analyses; results reported below).

Achieved means from 83 arms (35 trials), also included in the final dose-response analysis, were displayed in forest plots with their 95% CI and pooled weighted values estimated, both overall (pooled estimate: 57.9 nmol/L; 95%CI: 54.6-61.3) and by relevant subgroups (Appendix D.C, Figure 4, Figure 5 – Figure 15)

Mean differences in achieved mean serum 25(OH)D concentration were calculated for 30 RCTs, out of the final 35 studies included in the dose-response analysis, where a control/placebo group and at least one intervention group were available (i.e. 5 trials out of 35 did not have a control group[42]). In case of multiple intervention groups, the achieved mean serum 25(OH)D of the first intervention arm (with the lowest dose) was selected to be compared to the achieved mean serum 25(OH)D of the control group. The pooled weighted mean difference across the 30 trials was 29.3 nmol/L (95% CI 26.4–32.3) (Appendix D.D, Figure 16), with average achieved means of 41.3 nmol/L (SD = 10.3) and 70.8 nmol/L (SD = 14.1) in the control and intervention groups respectively and very close average baseline means (50.4 and 51.1 nmol/L, SD = 16). Analysis of weighted pooled estimate of mean differences in achieved mean serum 25(OH)D by 5 µg increase in total vitamin D intake (between 5 and 50 µg/day) is also reported in Appendix D.D (Figure 17).

Results from studies on specific populations (infants, lactating and pregnant women) were not included in separated meta-analyses (Appendix D.A) because their number (two arms on pregnant women, three arms on lactating women, three arms on infants) and characteristics were not deemed suitable (a minimum of three per sub-population is requested); their results are addressed narratively in the contractor's report.

8. Meta-regression of the response of serum 25-hydroxyvitamin D to total vitamin D intake

Weighted linear meta-regression analyses of total vitamin D intake (i.e. habitual intake of the vitamin plus the supplemental dose) versus mean achieved serum or plasma 25(OH)D concentration measured at the end of the winter sampling points were performed.

The models were developed applying a random-effects approach ('random-effects meta-regression'), in which the extra variability due to heterogeneity is incorporated in the same way as in a random-

[41] Indicated as "first author date a" or "first author date b" or "first author date c" in case two (or three) different populations were included in the same study, e.g. normal weight, overweight and obese people.
[42] Barger-Lux et al., 1998, DeLappe et al., 2006, Goussous et al., 2005, Pekkarinen et al., 2010, and Vieth et al., 2001.

effects meta-analysis, where the influence of more precise studies on the relationship is mitigated by the consideration of variability across studies. The approach allowed for extra residual heterogeneity among dose-response estimates not modelled by the explanatory variables identified and tested.

8.1. Studies included in the dose-response analysis

Meta-regression analyses were performed on the final data set (83 arms, 35 trials), as identified in section 8.

Most of the exclusions from the preliminary data set were based on inconsistencies in achieved means, mean differences (between intervention and control in the same trial) and net mean changes (between baseline and achieved mean in the same arm) of serum 25(OH)D (in the same trial across intervention groups and/or across trials in the same dose group). Careful re-consideration of study characteristics (e.g. design, type of participants, supplementation scheme, reporting issues, and summary data type) was the basis as to whether confirm exclusion of the identified arms (or entire related trial) (Appendix D.A, Table 8 – fourth column).

In addition, four arms were excluded based on model checking results (statistical outliers), after revision of all standardised residuals that were found to be either smaller than - 2 or larger than + 2. Two further exclusions were applied after re-consideration of the maximum supplemented vitamin D dose to be included, i.e. 50 µg/day, in order to model total vitamin D intakes that were not exceeding 100 µg/day (the UL set by EFSA) (Appendix D.A, Table 85 – fourth column).

8.2. Model construct

Two different model constructs of the dose-response relationship between plasma/serum 25(OH)D and total vitamin D intake were explored:

Log-linear: total vitamin D intake was transformed to the natural log (Ln) before regression analysis; the regression intercept was set to 0 nmol of mean achieved 25(OH)D serum level to prevent negative values (which are biologically implausible). The intercept of the final adjusted model was not statistically significantly different from zero.

Linear: mean achieved serum 25(OH)D concentrations were regressed to total vitamin D intake on its original scale; the total vitamin D intake data points modelled were limited by a maximum intake dose of 35 µg/day, on the basis of evidence showing that the slope response of serum 25(OH)D to increasing dose becomes constant at such dose, as suggested by others (Aloia et al., 2008).

A non-linear response of serum 25(OH)D to vitamin D intake was expected due to metabolic kinetics (Heaney et al., 2008); in fact, the response of serum 25(OH)D is not best described by a linear fit model at doses above 35 µg/day.

The interest in exploring the linear model construct as an alternative to the curvilinear one was that the latter has a steep decline in achieved serum 25(OH)D concentrations particularly at the lower end of the range of total vitamin D intakes, and at zero intake the achieved serum 25(OH)D is forced to be 0 nmol/L to avoid a negative predicted value.

The WG decided to retain the log linear construct to better describe the dose-response shape and to be able to include results from higher dose trials (i.e. up to 50 µg/day).

8.3. Model fitting

For each random-effects meta-regression model the statistics T^2 (*tau-squared*, between-study variance) and Adjusted R^2 were calculated. T^2 was estimated using the restricted maximum likelihood

method (Thompson and Sharp, 1999) with Knapp-Hartung modification of the estimate of variance-covariance matrix of the regression coefficients (Knapp and Hartung, 2003) to reduce false-positive rates.

The change in T^2 after inclusion of each covariate gives the amount of heterogeneity explained by the fitted model, and this value over the T^2 from the null model gives the proportion of between-study variance explained (Adjusted R^2).

T^2 decreased from 312 to 46 in the final model, with included factors explaining up to 85% of heterogeneity (Appendix D.E, Table 13), i.e. ((312-46)/312)*100 = 85% (Adjusted R^2) of between-study variance explained and 15% of unexplained heterogeneity.

The residual I^2 statistics gives a measure of the percentage of the residual variation (the one not explained by the covariates) that is attributable to between-study heterogeneity.

Residual I^2 also decreased after inclusion of the final set of covariates, yet remaining quite high (87%) (Appendix D.E, Table 13).

In addition to the evaluation of the relative reduction of T^2 and of the joint testing (using the F distribution) of covariates as introduced in the model, a backward elimination process was used to check the set of explanatory variables identified by manual fitting in the final model as significant predictors of the mean achieved serum levels.

8.4. Baseline measurements

The influence of the mean baseline 25(OH)D concentration on the dose-response relationship was described by plotting its values against the corresponding achieved mean values and explored in subgroup analyses (Appendix D.C, Figure 6 ≤ versus > 50 nmol/L) and meta-regression models (continuous covariate, Table 5). Bubble plots of net values (achieved 25(OH)D concentrations minus baseline values) were also considered to complement the dose-response analysis (not shown in this report).

After total vitamin D intake, the mean baseline 25(OH)D concentration was the factor explaining the highest proportion of between-study variability (17% in the simple meta-regression model – not shown in this report).

This is not surprising as it is likely that baseline values can serve as a surrogate for many influencing factors, potentially including some of those that could not be measured in the analysed trials. In fact, in the final adjusted model, the regression coefficient for the mean baseline was only marginally changed by the mutual adjustment for all the other included covariates (0.53 vs 0.48, (Appendix D.E, Table 13)).

8.5. Inter-individual variability on dietary intake

Previous analyses on vitamin D intake-status have encountered difficulties in taking into account the inter-individual variability on intake required to reach a chosen serum 25(OH)D cut-off.

The CI in meta-regression analyses provides an estimate of the uncertainty about the fitted response line due to sampling, but does not provide any estimate of the variability between individuals in terms of dietary intake of vitamin D needed to achieve a serum 25(OH)D concentration.

Attempts have been made to augment the meta-analytic approach by using individual data from vitamin D RCTs (Cashman et al., 2011b), which was not possible in the case of the current analysis as no individual data were available.

8.6. Model checking diagnostics

Outliers and influential studies were detected and tests for normality and homoscedasticity carried out to check for model assumptions (e.g. normality of the random effects).

The normal probability plot of the standardised predicted random effects did not show substantial departure from normality; outliers were identified by evaluation of standardised residual values smaller than - 2 or larger than + 2 (Appendix D.A, Table 8, fourth column) as estimated from the final models.

When several covariates are used in meta-regression, either in several separate simple meta-regressions or in one multiple meta-regression, there is an increased chance of at least one false-positive finding (type I error). The statistics obtained from the random permutations can be used to adjust for such multiple testing by comparing the observed t statistic for every covariate with the largest t statistic for any covariate in each random permutation (Higgins and Thompson, 2004).

Permutation-based p-values were calculated by running a Monte Carlo permutation test.

8.7. Dose-response influencing factors, investigation of heterogeneity between studies

A number of factors potentially influencing the dose-response relationship were identified *a priori* both from the relevant literature and upon feedback from the WG.

The following list was prioritised based on the outcome of WG's discussions; a selection of priority study-level characteristics was tested in independent subgroup analyses and incorporated in the meta-regression models one at a time and in the final multivariable model:

- *Total vitamin D intake*: as continuous, as categorical (cut-offs determined by an increment of 5 µg/day; Appendix D.C, Figure 7),
- *Baseline serum concentration*: as continuous, as dichotomous (cut-offs: 30 nmol/L (not shown in this report) and 50 nmol/L (Appendix D.C, Figure 6),
- *Study duration*: ≤ three months vs > three months,
- *Latitude*: as categorical, stratified by > 40°N to < 50°N and ≥ 50°N and 78°S[43],
- *Assay method used*: HPLC and LC-MS versus immunoassays (i.e. RIA, CBPA, ELISA),
- *Period of study publication*: also related to trends in analytical methods (cut-off: year 2000) (not shown in this report),
- *Body Mass Index*: a 'proxy' for body composition (which is not reported in the included trials); as continuous (study-level mean BMI), as per four categories: "Normal weight", "Overweight", "Obese", "Not reported" (Appendix D.C, Figure 13),
- *Ethnicity*: a 'proxy' for skin pigmentation and some lifestyle habits that were usually not reported in the included trials; as per four categories: "Caucasian", "African", "Mixed", "Not Reported",
- *Co-supplemented calcium*: as categorical (Yes, No/Unknown) (not shown in this report)
- *Funding source*: as categorical ("Non-profit", "Profit", "Mixed", "Not reported") (not shown in this report),

[43] Only one trial (four arms) was undertaken in the Southern hemisphere (at 78°S). All the other trials included were undertaken in the Northern hemisphere (41°N – 63°N).

- *Age*: as continuous (study-level mean age), as categorised according to three population groups (children, adults, older adults; the latter from trials where the reported or estimated mean age was ≥ 60 years) (Appendix D.C, Figure 14)
- *Sex*: as categorical based on % of males ("Both" for studies on mixed populations, "Women" for studies on women only, "Men" for studies on men only)

Risk of bias dimensions: all individually categorised as "Yes", "No/Unknown" (adequate randomisation, adequate allocation concealment, adequate blinding description, compliance assessed, drop-outs addressed, dose check reported); as combined by the contractor in an overall RoB assessment ("High", "Moderate", "Low" RoB) (Appendix D.B, Table 12).

The following further categorisations were also applied and tested a posteriori:

- Duration: ≤ 3 mo. vs > 3 months & < 6 months vs 1–2 years (Appendix D.C, Figure 8),
- Latitude: < 50°N, 50–55°N, > 55°N. For 76% of arms latitude was > 50°N **(**Appendix D.C, Figure 9),
- Assay method used: RIA versus HPLC versus LC-MS versus CPBA versus ELISA & Not Reported versus Other (Appendix D.C, Figure 11). In the final model (Section 1.9.8.), each analytical method was retained as an individual category to be able to estimate the specific effects,
- Ethnicity: "Caucasian" "Mixed" "Not Reported". "African" was grouped to the "Mixed" category, as it included three arms only (Appendix D.C, Figure 12).

Study start period was subsequently considered instead of publication year as a better proxy to the temporal trends in assay method use (as continuous - since year of first study in analysis, i.e. 1985; as dichotomous -before or after 2000) (Appendix D.C, Figure 10).

Pooled estimates in the placebo/control arms and intervention arms were also reported for descriptive purposes (Appendix D.C, Figure 5).

All results (Appendix D.C, Figure 4–Figure 15) were interpreted only qualitatively and group summary estimates compared by visual inspection; sub-group comparisons are observational in nature and results from statistical testing should not be used to infer that estimates differ from one stratum to another.

8.9. Derivation of DRVs

The meta-regression analysis carried out on the selected arms resulted in two predictive equations of achieved serum 25(OH)D:

y = 23.2 Ln (total vitamin D intake) (unadjusted model) (Appendix D.F, Figure 18) and

y = 16.3 Ln (total vitamin D intake) adjusted for baseline concentration (continuous; µg/day), latitude (continuous; °N), study start year (continuous; years since first study in analysis - 1985), type of analytical method applied (RIA, HPLC, LC-MS, CPBA, ELISA/not reported, Other), assessment of compliance (yes, no/unknown) (Table 5, and Appendix D.F, Figure 19).

Age and sex were not included in the final model as did not explained further neither within- nor between- study variability. The role of BMI was also tested in the subset of arms for which such information was available (83%); overweight and obese subgroups from the study populations showed on average higher achieved means when compared to the normal weight group (Appendix D.C, Figure 13) but lower values once adjusted for all other covariates. **BMI was not included in the final model** as it did not reach statistical significance in the preliminary analyses from the preliminary data set (116 arms) and in consideration of potential ecological fallacy (i.e. associations with mean BMI values when available or calculated from mean height and mean weight at study-level are not necessarily consistent with associations with individual-level BMI values).

Table 5: Adjusted meta-regression model (outcome variable: mean achieved 25(OH)D in nmol/L; n = 83)

Covariate	*β Coefficient*	*SE*	*P > z*	*95% CI*	
Ln of Total vitamin D intake - µg/day	16.33	0.94	< 0.001	14.45	- 18.21
Mean Baseline 25(OH)D - nmol/L	0.50	0.05	< 0.001	0.39	- 0.61
Latitude - °N	- 0.46	0.09	< 0.001	- 0.63	- - 0.29
Study start year (years since 1985)	0.93	0.21	< 0.001	0.51	- 1.35
Assay					
*RIA**	0.00				
HPLC	- 1.93	3.29	0.56	-8.49	- 4.62
LC-MS	- 4.72	3.00	0.12	-10.69	- 1.26
CPBA	0.63	3.86	0.87	-7.07	- 8.33
ELISA/nr	- 6.40	2.68	0.02	-11.73	- - 1.06
Other	1.30	3.61	0.72	-5.89	- 8.49
Compliance assessed					
*Yes**	0.00				
No/unknown	7.79	2.97	0.01	1.86	- 13.71

* reference category SE: standard error

P > z: indicates the probability of the hypothesis that the beta-coefficient = 0 (since p = 0.05 is conventionally assumed as the cut-off for statistical significance in the analysis, a p value lower than 0.05 provides good evidence that the beta-coefficient is significantly different from 0).

The same equations were used both to predict the achieved mean serum 25(OH)D levels conditional to total vitamin D intakes of 5, 10, 15, 20, 50, 100 µg/d (Table 6) and to estimate the total vitamin D intakes that would achieve serum 25(OH)D concentrations of 50, 40, 30, 25 nmol/l (Table 7).

All values were calculated by using the regression equations of the predicted mean, of the lower and upper limits of the 95% CI of the predicted mean and of the lower and upper limits of the 95% prediction interval (PI) of the predicted mean. In the adjusted multivariable models all covariates were set to their mean values (Mean Baseline 25(OH)D: 50.7 nmol/L; Latitude: 53°N; Study start year: 2005; Assay – HPLC: 10%; LC-MS: 18%; CPBA: 13%; ELISA: 20%; Other: 8%; Compliance not assessed/unknown: 27%).

A stratified analysis was carried out to quantify the impact of the exclusions of the four trials on children (nine arms) on the predicted achieved mean serum 25(OH)D levels (Appendix D.G, Table 14, ADULTS estimates) and estimated total vitamin D intakes (Appendix D.G, Table 15, ADULTS estimates). In the restricted dataset (74 arms) there was an overall small decrease in all serum estimates (and consequently a small increase in total intakes that would achieve target values); this is possibly due both to the fact that 'children' arms were just 9 and that children tend to achieve the same levels as the adults at a lower total intake (Appendix D.G, Table 14, CHILDREN estimates). Overall estimates did not substantially change as compared to the full data set including children.

Values based only on the 4 children trials were not calculated in the fully adjusted meta-regressions, as they would have required a much higher minimum number of 'points' per covariate (at least 10 arms for each included factor); instead, values from a model adjusted by mean baseline 25(OH)D were provided. As such these estimates are not directly comparable to the adults' ones, as they are not adjusted for the same set of covariates. The unadjusted model showed lower average intakes, but estimates were much less precise (with 95% CI overlapping to those from the adults data), and could only be evaluated qualitatively (Appendix D.G, Table 15, CHILDREN estimates).

In the meta-analytic context, when a random-effects approach is applied, the CI reflects the precision with which we estimate the pooled (across studies) mean effect size (via the available

sample of studies), while the PI reflects the actual dispersion of the true effects around the mean effect size.

If, for instance, we have estimated a mean response of 50 with a CI of 40 to 60, we know that the range of 40 to 60 includes with a certain frequency (conventionally 95% of the times) the true *mean response* in the population of studies from which the sample was drawn.

From a related PI of 30 to 70, we can tell that probably (conventionally 95% of the times) such range will include the *true effect in a new study from the same population of studies.* If the number of studies were infinite, then the CI width would approach zero but the PI would show little change.

When interpreting the intervals drawn around the meta-regression lines, the **CI illustrates our uncertainty about the position of the line** (i.e. across-study conditional means), **while the PI illustrates our uncertainty about the *true mean effect we would predict in a future study*** (i.e. the dispersion of the true effects around their mean).

As such it is possible to think of the latter only as an approximation of the interval that would allow for estimation of the requirements for 95% of the population, as it refers to the population of *mean* responses (not *individual* responses) as analysed in the random-effects model.

6184 **Table 6:** Predicted achieved serum 25(OH)D at selected values of total vitamin D intake

Regression equations used to predict serum 25(OH)D	Predicted serum 25(OH)D at selected values of total vitamin D intake					
	100 µg/day	50 µg/day	20 µg/day	15 µg/day	10 µg/day	5 µg/day
Unadjusted models						
y = 23.2 Ln (total vitamin D intake) §						
Predicted mean	**107**	**91**	**69**	**63**	**53**	**37**
95% CI lower limit	**101**	**86**	**66**	**59**	**50**	**35**
95% CI upper limit	**113**	**96**	**73**	**66**	**56**	**39**
95% PI lower limit	**78**	**62**	**41**	**34**	**25**	**9**
95% PI upper limit	**136**	**119**	**98**	**91**	**82**	**66**
Adjusted models ł						
y = 16.3 Ln (total vitamin D intake) + 0.5 mean baseline 25(OH)D - 0.5 latitude + 0.9 start year - 2.0 HPLC - 4.7 LC-MS + 0.6 CPBA - 6.4 ELISA/nr + 1.3 Other assay + 7.8 Compliance not assessed §						
Predicted mean	**94**	**83**	**68**	**63**	**57**	**45**
95% CI lower limit	**89**	**78**	**63**	**58**	**52**	**40**
95% CI upper limit	**100**	**88**	**73**	**69**	**62**	**51**
95% PI lower limit	**80**	**69**	**54**	**49**	**42**	**31**
95% PI upper limit	**109**	**98**	**83**	**78**	**71**	**60**

6185
6186 CI, confidence interval; PI, prediction interval.
6187 § Predicted mean regression equations are reported (y = mean achieved serum 25-hydroxyvitamin D).
6188 ł **Estimates from the adjusted models are based on all covariates set to their mean values.**

Table 7: Estimated vitamin D intakes at selected serum 25(OH)D cut-off values

Regression equations used to estimate vitamin D intake	Estimated vitamin D intake at selected serum 25(OH)D cut-off values			
	50 nmol/L	40 nmol/L	30 nmol/L	25 nmol/L
Unadjusted model				
y = 23.2 ln (total vitamin D intake) §				
Predicted mean	8.7	5.6	3.6	2.9
95% CI lower limit	9.8	6.2	3.9	3.1
95% CI upper limit	7.7	5.1	3.4	2.8
95% PI lower limit	29.9	19.4	12.6	10.1
95% PI upper limit	2.5	1.7	1.1	0.9
Adjusted model ł				
y = 16.3 ln (total vitamin D intake) + 0.5 mean baseline 25(OH)D - 0.5 latitude + 0.9 start year - 2.0 HPLC - 4.6 LC-MS + 0.5 CPBA - 6.9 ELISA/nr + 1.3 Other assay + 7.8 Compliance not ass. §				
Predicted mean	6.6	3.6	1.9	1.4
95% CI lower limit	9.1	4.9	2.7	2.0
95% CI upper limit	4.8	2.6	1.4	1.0
95% PI lower limit	16.1	8.7	4.7	3.5
95% PI upper limit	2.7	1.5	0.8	0.6

CI, confidence interval; PI, prediction interval.

§ Predicted mean regression equations are reported (y = mean achieved serum 25-hydroxyvitamin D).

ł **Estimates from the adjusted model are based on all covariates set to their mean values.**

9. Quality of the body of evidence: addressing risk of bias

The rating by the contractor of individual trials in terms of RoB (individual dimensions and overall assessment) was used to evaluate whether heterogeneity of results could be attributed to differences in internal validity, both in the meta-analyses and meta-regression models (Appendix D.B, Table 12). The following approaches were discussed and applied accordingly:

- To run the analysis on low-moderate-risk trials only (restriction): this option could not be applied as the proportion of low-risk arms was only 16% (plus moderate-risk ones accounting for an additional 18%). The trade-off between bias and precision would have been too much towards (possibly) more valid but less precise estimates;

- To run a sensitivity analysis and see how the response changes if high-risk studies are excluded: this was not carried out considering that the majority of trials were rated high-RoB;

- To run a subgroup analysis (or meta-regression) re-grouping the RoB variable into a dichotomous one: this was considered but the covariate was tested as originally coded (low, moderate, high risk). The lack of a statistically significant difference between studies at high and low RoB (data not shown in this report) should be interpreted cautiously as meta-regression analyses are observational in nature;

- To use individual dimensions as recorded by the contractor: each RoB dimension was evaluated in univariate and multivariable analyses. Assessed compliance (categorised as yes versus no/unknown and independently of its definition across trials) was found to play a role in further explaining the variability between studies (Appendix D.E, Table 13); all others dimensions (randomization appropriate, allocation concealment, etc.) were not statistically significantly impacting on the estimates (not shown in this report);

- To integrate a qualitative (narrative) evaluation of RoB in the discussion of the analysis results.

10. Sensitivity Analyses

A number of sensitivity analyses were carried out to evaluate whether the findings were robust to the assumptions made in the systematic review protocol and the analyses (e.g. meta-regression models).

When sensitivity analyses show that the overall result and conclusions are not substantially affected by the different decisions that could be made during the review process, the results of the review can be regarded with a higher degree of certainty.

There were a number of assumptions/decisions/issues provisionally identified that could potentially be tested in sensitivity analyses by comparing the results obtained with alternative input parameters to those from the default model or by restricting to specific sub-sets; none of them raised serious concerns about the robustness of the overall analysis (the most substantial departures were detected in the smallest, then less representative, subsets of the final data set).

The following analysis were considered:

- On data cleaning issues: implausible values, missing data,
- On quality dimensions*:* compliance assessment,
- On analytical approaches: data imputation; cut-off points, choice of categories,
- On eligibility criteria: fortified food trials; range of doses (exclusion of doses higher than 100 µg/day); characteristics of participants (exclusion of non-healthy volunteers, of supplement users, etc.; Appendix D.H, Table 16).

11. Observational studies: contribution of their results to the analysis

Meta-analyses were performed separately for RCTs and observational studies (prospective cohort studies) on the basis that, in principle, evidence from randomised and non-randomised studies is not considered comparable. Eight prospective observational studies from seven articles were included. (Appendix D.I, Table 17). They represented 11 study groups (e.g. children versus adults in Andersen 2013, Caucasian group versus Asian group in Darling et al. (2013), Caucasian from one study centre versus a group of Caucasian and a group of Asian people in another study centre in MacDonald et al. (2011)), three of which were on children (mean age between 11 and 16 years).

Achieved mean serum 25(OH)D concentration (and 95% CI) was investigated by study group (Appendix D.I, Figure 20), as well as by relevant sub-groups: age (children versus adults; Appendix D.I, Figure 21:)), baseline mean serum 25(OH)D concentrations (≤ versus > 50 nmol/L; Appendix D.I, Figure 22) and latitude (< 50 °N versus ≥ 50 °N; Appendix D.I, Figure 23).

12. Publication bias

Several systematic reviews of empirical studies have found that studies with statistically significant or positive results are more likely to be published than those with non-significant or negative results. Investigators' decisions not to submit papers with negative results for publication, rather than editors' rejection of such papers, tend to be the main source of publication bias. Studies with statistically significant results also tend to be published earlier than studies with non-significant results. If studies are missing from a systematic review for these reasons, effects may be over-estimated (Higgins et al., 2011).

Publication bias was examined by inspecting funnel plots (Sterne and Egger, 2001) and by performing the Egger's test for funnel plot asymmetry (Egger et al., 1997) on mean differences in achieved mean serum 25(OH)D from the 30 RCTs included in the meta-analyses (see Section 8.).

Egger's test performs a linear regression of the intervention effect estimates on their standard errors, weighting by 1/(variance of the intervention effect estimate) (Appendix D.J, Figure 24); the test was not statistically significant (p = 0.149).

Funnel plots investigate the association between study size and effect size; there was no particular indication of funnel plot asymmetry, as trials testing a dose of 5-<10 µg/day were missing in the right-hand side of the funnel while trials testing 45 µg/day and more were missing in the left-hand side (Appendix D.J, Figure 25).

13. Uncertainty analysis

Sources of uncertainty and their potential impact on the final estimates, where possible, were identified and discussed:

- General interpretation of meta-regression results – the associations derived from meta-regressions are observational and have a weaker interpretation than those derived from randomized comparisons; this applies especially when population characteristics are included as means at study level,

- Inter-individual variability on intake - failure to account for it may lead to underestimation of the predicted intake of vitamin D needed to maintain a specified serum 25(OH)D level (Cashman et al., 2011b),

- Predicted achieved mean serum 25(OH)D levels and estimated total vitamin D intakes calculated based on the 95% CI of the predicted mean from the adjusted models were less

accurate than those from the unadjusted ones, due to the approximation of the fitting on the pair wise limits,

- Predictions from the lower range of the total vitamin D intakes are less accurate than those for higher values because of the log-linear construct (not optimal fitting in that intake range),

- Ecological fallacy - key risk factors that vary across populations and that can be measured only as aggregate values, such as age, gender and BMI, are difficult to address adequately by meta-regression. One reason for this is that aggregated values tend to exhibit little between-study variation, thus providing minimal information across the potential range of the factor. Use of aggregated values may also introduce bias because of the failure to account for the within-study variation (Thompson and Higgins, 2002),

- Selection of RCTs/arms – the main objective of the additional exclusion of arms from the final data set was to try to 'remove' as much heterogeneity as possible that could be attributable to differences in design, bias, and/or methods, so that only "clinical" heterogeneity (i.e. between-study variability due to population's features) would be left to be modelled and characterised. It is difficult to quantify the potential relative misclassification due to such a selection; the proportion of heterogeneity explained by the influencing factors in the final subset was higher than that in the preliminary data set (85% vs 56%) but the regression coefficients of all covariates were almost unchanged. This could be interpreted as a relative reduction of heterogeneity more in its methodological component across included studies, due to the nature of the criteria applied for the additional exclusions.

Appendix D. Dose-response analysis undertaken by EFSA of serum 25(OH)D to total vitamin D intake: methods and key results: appendices

A. *LIST OF TRIALS ARMS NOT INCLUDED IN THE META-ANALYSES AND DOSE-RESPONSE ANALYSIS.*

Table 8: Reasons for exclusions from preliminary data set and final data set (58 arms out of 141).

RCT arms	Suppl. vitamin D dose (µg/day)	Reasons for exclusion from preliminary set (25 arms)	Reasons for exclusion from final set (33 arms)
(Ala-Houhala et al., 1986)a*	12.5	Study on pregnant women	-
(Ala-Houhala et al., 1986)a	0	Study on pregnant women	-
(Ala-Houhala et al., 1986)b*	50	Study on lactating women	-
(Ala-Houhala et al., 1986)b	25	Study on lactating women	-
(Ala-Houhala et al., 1986)b	0	Study on lactating women	-
(Ala-Houhala et al., 1986)c*	10	Study on infants	-
(Ala-Houhala et al., 1988b)	10	Study with supplemented vitamin D_2	-
(Ala-Houhala et al., 1988b)	0	Study with supplemented vitamin D_2	-
(Atas et al., 2013)	10	Study on infants	-
(Atas et al., 2013)	5	Study on infants	-
(Barger-Lux et al., 1998)	1250	Arm with supplemented dose > 100 µg/day	-
(Barger-Lux et al., 1998)	250	Arm with supplemented dose > 100 µg/day	-
(Brazier et al., 2002)	20	-	Methodological considerations applicable to whole study
(Brazier et al., 2002)	0	-	Inconsistent net mean change + methodological considerations
(Close et al., 2013b)	125	Arm with supplemented dose > 100 µg/day	-
(Close et al., 2013b)	0	-	Inconsistent net mean change and achieved mean + methodological considerations
(Forman et al., 2013)	100	-	Arm with supplemented dose ≥ 100 µg/day
(Heaney, 2003)	250	Arm with supplemented dose > 100 µg/day	-
(Heaney, 2003)	125	Arm with supplemented dose > 100 µg/day	-
(Holick et al., 2008)	25	Arm with supplemented vitamin D_2	-
(Holick et al., 2008)	25	Arm with supplemented vitamin D_2	-
(Holm et al., 2008)	5	-	Supplementation scheme was 5 µg/3 days + inconsistent mean difference
(Holm et al., 2008)	0	-	Control group only left from study
(Honkanen et al., 1990)b	45	-	Methodological considerations applicable to whole study
(Honkanen et al., 1990)b	0	-	Statistical outlier

RCT arms	Suppl. vitamin D dose (µg/day)	Reasons for exclusion from preliminary set (25 arms)	Reasons for exclusion from final set (33 arms)
(Johnson et al., 2005)	15	-	Inconsistent achieved mean + methodological considerations
(Johnson et al., 2005)	0	-	Methodological considerations applicable to whole study
(Johnson et al., 2005)	0	-	Methodological considerations applicable to whole study
(Larsen et al., 2012)	25	-	Statistical outlier
(Larsen et al., 2012)	0	-	Control group only left from study
(Lehmann et al., 2013)	50	Arm with supplemented vitamin D_2	-
(Mocanu et al., 2009)	125	Study with supplemented dose > 100 µg/day	-
(Nelson et al., 2009)	20	-	Methodological considerations applicable to whole study
(Nelson et al., 2009)	0	-	Inconsistent net mean change + methodological considerations
(Patel et al., 2001)a	20	-	Inconsistent achieved mean + methodological considerations
(Patel et al., 2001)a	0	-	Methodological considerations applicable to whole study
(Patel et al., 2001)b	20	-	Inconsistent achieved mean + methodological considerations
(Porojnicu et al., 2008)	5	Quantitative data on response not available	-
(Porojnicu et al., 2008)	0	Quantitative data on response not available	-
(Rich-Edwards et al., 2011)	7.5	-	Statistical outlier (fortified UHT milk arm)
(Schmidt and Zirkler, 2011)	5	-	Inconsistent mean difference + methodological considerations
(Schmidt and Zirkler, 2011)	0	-	Control group only left from study
(Sorva et al., 1994)	25	Arm with supplemented vitamin D_2	-
(Sorva et al., 1994)	25	-	Statistical outlier
(Sorva et al., 1994)	0	-	Control group only left from study
(Vieth et al., 2001)	100	-	Arm with supplemented dose ≥ 100 µg/day
(White et al., 2009)	3	Mixed intervention **, very high baseline values	-
(White et al., 2009)	0	Mixed intervention **, very high baseline values	-
(White et al., 2009)	0	Mixed intervention **, very high baseline values	-
(Wood et al., 2014)_nw	25	-	Methodological considerations applicable to whole study

RCT arms	Suppl. vitamin D dose (µg/day)	Reasons for exclusion from preliminary set (25 arms)	Reasons for exclusion from final set (33 arms)
(Wood et al., 2014)_nw	10	-	Methodological considerations applicable to whole study
(Wood et al., 2014)_nw	0	-	Inconsistent baseline mean value + methodological considerations
(Wood et al., 2014)_ow	25	-	Methodological considerations applicable to whole study
(Wood et al., 2014)_ow	10	-	Methodological considerations applicable to whole study
(Wood et al., 2014)_ow	0	-	Inconsistent baseline mean value + methodological considerations
(Wood et al., 2014)_ob	25	-	Methodological considerations applicable to whole study
(Wood et al., 2014)_ob	10	-	Methodological considerations applicable to whole study
(Wood et al., 2014)_ob	0	-	Inconsistent baseline mean value + methodological considerations

*e.g. (Ala-Houhala et al., 1986)a, (Ala-Houhala et al., 1986)b and (Ala-Houhala et al., 1986)c (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study, but different population groups (e.g. in this case: pregnant women, lactating women and infants).

** Food fortified with vitamin D + training exercise, compared to supplements without vitamin D +training exercise.

nw, normal weight; ob, obese; ov, overweight; UHT, Ultra-high temperature.

6310 *B. TRIALS INCLUDED IN THE DOSE-RESPONSE ANALYSIS (35 TRIALS) – MAIN STUDY CHARACTERISTICS*

6311 **Table 9:** Country, latitude, age, sex, duration (35 trials)

Source	Country	Latitude	Mean age	Age range	Males	Duration
		°N	*years*	*years*	*%*	*weeks*
(Barger-Lux et al., 1998)	USA	41.2	28	20–37	100	8
(Barnes et al., 2006)	IE	54.8	22	18–27	50	8
(Bischoff et al., 2003)	CH	47.3	85	-	0	12
(Bolton-Smith et al., 2007)	UK	56.3	70	60+	0	104
(Bonjour et al., 2013)	FR	50.7	86	60+	0	8
(Braam et al., 2003)	NL	50.9	55	50–60	0	156
(Cashman et al., 2008)	IE	51	30	20–40	50	22
(Cashman and Kiely, 2009)	IE	51	71	64+	40	22
(Cashman et al., 2012)	IE	51	57	50+	38	10
(Cashman and Kiely, 2014)	IE	51	60	50+	28	15
(de Gruijl and Pavel, 2012)	NL	52.2	24	18–30	9	8
(DeLappe et al., 2006)	IE	53.2	80	-	0	13
(Forman et al., 2013)	USA	42.2	51	30–79	35	13
(Goussous et al., 2005)	USA	42.2	65	50+	27	13
(Hansen et al., 2010)	NO	60.4	35	20–60	100	23
(Harris and Dawson-Hughes, 2002)a	USA	42	26	18–35	100	8
(Harris and Dawson-Hughes, 2002)b	USA	42	70	62–79	100	8
(Heaney, 2003)	USA	41.2	39	-	100	20
(Heikkinen et al., 1998)	FI	62.9	51	47–56	0	52
(Holick et al., 2008)	USA	42.3	60	18–84	31	6
(Honkanen et al., 1990)a	FI	63	70	67–72	0	11
(Hower et al., 2013)	DE	51.2	4	2–6	56	20
(Keane et al., 1998)	IE	53.2	78	65–92	24	47
(Lehmann et al., 2013)	DE	51.47	43	19–67	33	8
(Madsen et al., 2013)a	DK	55.7	10	4–17	48	26
(Madsen et al., 2013)b	DK	55.7	36	18–60	50	26
(Meier et al., 2004)	DE	50	54	33–78	33	25
(O'Connor et al., 2010)	DK	55.4	11	11–12	0	52
(Pekkarinen et al., 2010)	FI	61	74	69–79	0	52
(Rich-Edwards et al., 2011)	MN	48	10	9–11	53	7
(Smith et al., 2009)	AQ	78*	43	-	75	22
(Trautvetter et al., 2014)	DE	50.6	42	-	40	8
(Vieth et al., 2001)	CA	43	41	-	33	8
(Viljakainen et al., 2006c)	FI	61	71	65–85	0	12
(Viljakainen et al., 2009)	FI	61	29	21–49	100	26

6312 * Latitude of 78°S
6313 AQ, Antarctica; CA, Canada; CH, Switzerland; DE, Germany; DK, Denmark; FI, Finland; FR, France; IE, Ireland; MN,
6314 Mongolia; NL, the Netherlands; NO, Norway; UK, United Kingdom; USA, United States of America.
6315 e.g. (Madsen et al., 2013)a and (Madsen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study,
6316 but different population groups (e.g. in this case: children and adults).
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6319 **Table 10:** Start year, funding, ethnicity, analytical method, Ca co-supplementation (35 trials)

Source	Start year	Funding	Ethnicity	Analytical method	Ca Co-suppl.
(Barger-Lux et al., 1998)	1997	Mixed	Mixed	HPLC	No/unknown
(Barnes et al., 2006)	2005	-	-	ELISA	Yes
(Bischoff et al., 2003)	1999	Mixed	-	RIA	Yes
(Bolton-Smith et al., 2007)	2003	Mixed	-	RIA	Yes
(Bonjour et al., 2013)	2010	Profit	-	ELISA	Yes
(Braam et al., 2003)	1997	Mixed	Caucasian	RIA	Yes
(Cashman et al., 2008)	2006	Non-profit	Caucasian	ELISA	No/unknown
(Cashman and Kiely, 2009)	2007	Non-profit	Caucasian	ELISA	No/unknown
(Cashman et al., 2012)	2011	Mixed	Caucasian	ELISA	No/unknown
(Cashman and Kiely, 2014)	2012	Non-profit	Caucasian	LC-MS	No/unknown
(de Gruijl and Pavel, 2012)	2010	Mixed	Mixed	RIA	No/unknown
(DeLappe et al., 2006)	2003	-	-	RIA	Yes
(Forman et al., 2013)	2007	Mixed	African	RIA	Yes
(Goussous et al., 2005)	2003	Mixed	Mixed	RIA	Yes
(Hansen et al., 2010)	2008	Non-profit	Mixed	RIA	No/unknown
(Harris and Dawson-Hughes, 2002)a	2000	Mixed	-	CPBA	No/unknown
(Harris and Dawson-Hughes, 2002)b	2000	Mixed	-	CPBA	No/unknown
(Heaney, 2003)	2001	Non-profit	-	Other	No/unknown
(Heikkinen et al., 1998)	1990	Mixed	-	CPBA	Yes
(Holick et al., 2008)	2007	Mixed	Mixed	LC-MS	No/unknown
(Honkanen et al., 1990)a	1985	Mixed	-	CPBA	Yes
(Hower et al., 2013)	2010	Profit	Caucasian	Other	No/unknown
(Keane et al., 1998)	1993	Profit	-	CPBA	No/unknown
(Lehmann et al., 2013)	2012	Non-profit	-	LC-MS	No/unknown
(Madsen et al., 2013)a	2010	Mixed	-	LC-MS	No/unknown
(Madsen et al., 2013)b	2010	Mixed	-	LC-MS	No/unknown
(Meier et al., 2004)	2002	-	-	RIA	Yes
(O'Connor et al., 2010)	2008	Non-profit	Mixed	HPLC	No/unknown
(Pekkarinen et al., 2010)	2006	Non-profit	Caucasian	HPLC	Yes
(Rich-Edwards et al., 2011)	2009	Mixed	Mixed	LC-MS	No/unknown
(Smith et al., 2009)	2007	Non-profit	Caucasian	RIA	No/unknown
(Trautvetter et al., 2014)	2011	Profit	-	ELISA	Yes
(Vieth et al., 2001)	2000	Profit	Mixed	RIA	No/unknown
(Viljakainen et al., 2006c)	2002	Non-profit	-	HPLC	No/unknown
(Viljakainen et al., 2009)	2007	Non-profit	Caucasian	Other	No/unknown

6320 Ca Co-suppl, calcium co-supplementation; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid
6321 chromatography; LC-MS, liquid chromatography-mass spectroscopy; RIA, radioimmunoassay.
6322 e.g. (Madsen et al., 2013)a and (Madsen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study,
6323 but different population groups (e.g. in this case: children and adults).
6324

6325 **Table 11:** Vitamin D intakes, summary data (mean response with standard deviation) and body mass index (BMI) (35 trials, 83 arms)

Source	Habitual vitamin D intake	Supplemental Vitamin D dose	Total vitamin D intake	Participants per arm	Baseline Mean 25(OH)D	Baseline 25(OH)D SD	Achieved Mean 25(OH)D	Achieved 25(OH)D SD	Mean BMI
	µg/day	*µg/day*	*µg/day*	*n*	*nmol/L*	*nmol/L*	*nmol/L*	*nmol/L*	*kg/m²*
(Barger-Lux et al., 1998)	5	25	30.0	13	67	25	96	18	25.7
(Barnes et al., 2006)	1.6	15	16.6	12	48	16	87	25	24.8
(Barnes et al., 2006)	2.4	0	2.4	15	56	19	48	17	22.9
(Bischoff et al., 2003)*	3.3	20	23.3	62	36	24	66	25	24.7
(Bischoff et al., 2003)	3.3	0	3.3	60	35	24	32	12	24.7
(Bolton-Smith et al., 2007)	5.9	10	15.9	49	62	17	71	16	26.1
(Bolton-Smith et al., 2007)	5.6	10	15.6	50	62	15	74	15	25.8
(Bolton-Smith et al., 2007)	5	0	5.0	56	57	15	49	13	26.2
(Bonjour et al., 2013)*	2.8	10	12.8	29	19	5	45	16	26.2
(Bonjour et al., 2013)	2.8	0	2.8	27	16	5	21	16	26.6
(Braam et al., 2003)*	3.2	8	11.2	56	57	18	62	15	25.1
(Braam et al., 2003)	3.2	8	11.2	46	56	14	62	11	25.5
(Braam et al., 2003)	3.2	0	3.2	60	51	14	56	13	26.1
(Cashman et al., 2008)	3.6	15	18.6	53	74	25	71	19	26.1
(Cashman et al., 2008)	3.5	10	13.5	57	73	27	60	14	26.1
(Cashman et al., 2008)	4.3	5	9.3	48	67	31	52	11	26.1
(Cashman et al., 2008)	3.4	0	3.4	57	73	27	39	13	26.1
(Cashman and Kiely, 2009)	4.8	15	19.8	48	55	23	75	21	28.9
(Cashman and Kiely, 2009)	4.2	10	14.2	53	56	22	70	18	28.9
(Cashman and Kiely, 2009)	4.1	5	9.1	48	55	23	56	18	28.9
(Cashman and Kiely, 2009)	4.7	0	4.7	55	61	27	42	21	28.9
(Cashman et al., 2012)	7.6	20	27.6	13	50	16	69	9	28.3
(Cashman et al., 2012)	6.5	0	6.5	16	43	13	41	11	28.3
(Cashman and Kiely, 2014)	4.4	20	24.4	27	54	25	80	19	26.7
(Cashman and Kiely, 2014)	4.4	0	4.4	28	58	17	42	15	26.7
(Cashman and Kiely, 2014)	4.4	20	24.4	34	54	22	74	15	26.7
(Cashman and Kiely, 2014)	4.4	0	4.4	32	54	17	41	16	26.7

Source	Habitual vitamin D intake	Supplemental Vitamin D dose	Total vitamin D intake	Participants per arm	Baseline Mean 25(OH)D	Baseline 25(OH)D SD	Achieved Mean 25(OH)D	Achieved 25(OH)D SD	Mean BMI
	µg/day	*µg/day*	*µg/day*	*n*	*nmol/L*	*nmol/L*	*nmol/L*	*nmol/L*	*kg/m²*
(de Gruijl and Pavel, 2012)*	2.7	25	27.7	37	58	18	93	20	22.4
(de Gruijl and Pavel, 2012)	2.7	0	2.7	33	62	24	55	21	22.3
(DeLappe et al., 2006)*	3.4	20	23.4	51	42	27	60	27	-
(Forman et al., 2013)*	4.5	50	54.5	65	36	24	87	24	31
(Forman et al., 2013)	4.5	25	29.5	56	41	22	74	22	31
(Forman et al., 2013)	4.5	0	4.5	64	41	24	38	24	31
(Goussous et al., 2005)	3.8	20	23.8	23	49	17	66	15	26.7
(Goussous et al., 2005)	4.6	20	24.6	29	48	16	64	16	30.9
(Hansen et al., 2010)*	6.7	7	13.7	15	48	15	60	16	-
(Hansen et al., 2010)	6.7	1	7.7	14	48	25	49	20	-
(Harris and Dawson-Hughes, 2002)a	1.8	20	21.8	13	60	16	82	12	25
(Harris and Dawson-Hughes, 2002)a	3.3	0	3.3	12	49	17	44	17	25.1
(Harris and Dawson-Hughes, 2002)b	3.5	20	23.5	14	62	16	84	19	29
(Harris and Dawson-Hughes, 2002)b	1.5	0	1.5	11	54	18	49	18	30
(Heaney, 2003)*	5.4	25	30.4	17	72	16	80	16	26.2
(Heaney, 2003)	5.4	0	5.4	16	70	24	60	24	26.2
(Heikkinen et al., 1998)*	8.2	7.5	15.7	17	28	12	38	8	24.8
(Heikkinen et al., 1998)	8.2	7.5	15.7	18	24	8	33	8	25.7
(Heikkinen et al., 1998)	8.2	0	8.2	18	28	13	25	8	24.7
(Holick et al., 2008)*	4.4	25	29.4	20	49	28	65	28	30
(Holick et al., 2008)	4.4	0	4.4	10	47	22	45	22	29.3
(Honkanen et al., 1990)a*	8.7	45	53.7	25	43	17	81	13	-
(Honkanen et al., 1990)a	8.7	0	8.7	26	36	12	23	12	-
(Hower et al., 2013)	1.9	7.1	9.0	39	67	25	65	24	-
(Hower et al., 2013)	1.9	0.1	2.0	24	58	22	44	19	-
(Keane et al., 1998)*	3.6	5	8.6	24	24	5	46	11	-
(Keane et al., 1998)	3.6	0.1	3.7	18	25	5	32	14	-
(Lehmann et al., 2013)	3.2	50	53.2	42	44	23	89	22	23.7
(Lehmann et al., 2013)	3.2	0	3.2	19	41	15	32	13	23.7

Source	Habitual vitamin D intake	Supplemental Vitamin D dose	Total vitamin D intake	Participants per arm	Baseline Mean 25(OH)D	Baseline 25(OH)D SD	Achieved Mean 25(OH)D	Achieved 25(OH)D SD	Mean BMI
	µg/day	*µg/day*	*µg/day*	*n*	*nmol/L*	*nmol/L*	*nmol/L*	*nmol/L*	*kg/m²*
(Madsen et al., 2013)a	2.3	7.9	10.2	154	75	17	68	4	-
(Madsen et al., 2013)a	2.2	0	2.2	167	76	20	43	5	-
(Madsen et al., 2013)b	2.4	5.4	7.8	201	76	20	66	4	-
(Madsen et al., 2013)b	2.2	0	2.2	204	73	22	41	6	-
(Meier et al., 2004)	3.2	12.5	15.7	27	75	29	88	20	26.1
(Meier et al., 2004)	3.2	0	3.2	16	77	23	51	21	26.2
(O'Connor et al., 2010)*	2.3	10	12.3	33	48	16	58	14	18.1
(O'Connor et al., 2010)	2.3	0	2.3	34	48	18	40	18	18.1
(Pekkarinen et al., 2010)	6.4	20	26.4	20	58	10	74	10	26.9
(Rich-Edwards et al., 2011)**	2.2	7.5	9.7	140	20	10	50	15	16.4
(Rich-Edwards et al., 2011)	2.2	7.5	9.7	109	17	7	52	15	16.5
(Rich-Edwards et al., 2011)	2.2	0	2.2	101	20	10	20	10	17
(Smith et al., 2009)	8.9	50	58.9	18	45	14	71	23	28
(Smith et al., 2009)	8.2	25	33.2	19	44	19	63	25	31
(Smith et al., 2009)	7.6	10	17.6	18	44	18	57	15	29
(Smith et al., 2009)	15.7	0	15.7	7	36	17	34	12	28
(Trautvetter et al., 2014)	6.2	10	16.2	20	46	20	70	20	25
(Trautvetter et al., 2014)	6.5	10	16.5	17	50	16	67	16	25
(Trautvetter et al., 2014)	6.5	0	6.5	19	59	30	48	30	24
(Vieth et al., 2001)	5.4	25	30.4	33	43	17	65	17	-
(Viljakainen et al., 2006c)	9.7	20	29.7	13	44	14	68	14	27.2
(Viljakainen et al., 2006c)	10.6	10	20.6	11	47	10	61	10	25.8
(Viljakainen et al., 2006c)	9.7	5	14.7	13	46	14	57	14	25.7
(Viljakainen et al., 2006c)	10.9	0	10.9	12	52	20	44	20	25.6
(Viljakainen et al., 2009)	8.6	20	28.6	16	62	14	90	14	24.4
(Viljakainen et al., 2009)	7.6	10	17.6	16	60	12	76	12	24.9
(Viljakainen et al., 2009)	6.6	0	6.6	16	65	19	52	19	24.8

* Trials for which habitual dietary intake was imputed from national survey data (age-, sex- specific); ** Rich-Edwards 2011 values were imputed from Madsen 2013 (children with same mean age). NB: e.g. (Madsen et al., 2013)a and (Madsen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study, but different population groups (e.g. in this case: children and adults). BMI, body mass index; SD, standard deviation.

6329 **Table 12:** Risk of bias (RoB) dimensions – adequacy of randomisation, compliance assessment,
6330 dose check, overall RoB classification (35 trials)

Source	Randomisation adequate	Compliance assessed	Dose check	Overall Risk of Bias
(Barger-Lux et al., 1998)	Yes	Yes	Yes	High
(Barnes et al., 2006)	No/unknown	No/unknown	No/unknown	High
Bischoff-Ferrari 2003	Yes	Yes	No/unknown	Moderate
(Bolton-Smith et al., 2007)	Yes	Yes	No/unknown	Moderate
(Bonjour et al., 2013)	Yes	Yes	Yes	Moderate
(Braam et al., 2003)	Yes	No/unknown	No/unknown	Moderate
(Cashman et al., 2008)	Yes	Yes	Yes	Low
(Cashman and Kiely, 2009)	Yes	Yes	Yes	Low
(Cashman et al., 2012)	Yes	Yes	Yes	Moderate
(Cashman and Kiely, 2014)	Yes	Yes	Yes	Low
(de Gruijl and Pavel, 2012)	Yes	Yes	No/unknown	High
(DeLappe et al., 2006)	No/unknown	Yes	No/unknown	High
(Forman et al., 2013)	Yes	Yes	No/unknown	High
(Goussous et al., 2005)	No/unknown	Yes	No/unknown	High
(Hansen et al., 2010)	No/unknown	No/unknown	No/unknown	High
(Harris and Dawson-Hughes, 2002)a	No/unknown	No/unknown	No/unknown	High
(Harris and Dawson-Hughes, 2002)b	No/unknown	No/unknown	No/unknown	High
(Heaney, 2003)	No/unknown	Yes	Yes	High
(Heikkinen et al., 1998)	Yes	No/unknown	No/unknown	High
(Holick et al., 2008)	No/unknown	Yes	Yes	High
(Honkanen et al., 1990)a	No/unknown	No/unknown	No/unknown	High
(Hower et al., 2013)	Yes	Yes	Yes	High
(Keane et al., 1998)	No/unknown	No/unknown	Yes	High
(Lehmann et al., 2013)	Yes	Yes	Yes	Low
(Madsen et al., 2013)a	Yes	Yes	Yes	High
(Madsen et al., 2013)b	Yes	Yes	Yes	High
(Meier et al., 2004)	No/unknown	Yes	No/unknown	High
(O'Connor et al., 2010)	No/unknown	Yes	No/unknown	High
(Pekkarinen et al., 2010)	No/unknown	Yes	No/unknown	High
(Rich-Edwards et al., 2011)	Yes	Yes	No/unknown	Moderate
(Smith et al., 2009)	No/unknown	Yes	Yes	High
(Trautvetter et al., 2014)	No/unknown	Yes	Yes	High
(Vieth et al., 2001)	Yes	Yes	No/unknown	High
(Viljakainen et al., 2006c)	No/unknown	No/unknown	No/unknown	High
(Viljakainen et al., 2009)	No/unknown	Yes	Yes	High

6331 e.g. (Madsen et al., 2013)a and (Madsen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study,
6332 but different population groups (e.g. in this case: children and adults).
6333

6334

C. *Forest plots of achieved mean serum 25(OH)D concentrations by relevant factors explored in the dose-response models (random-effects meta-analyses) (35 trials, 83 arms)*

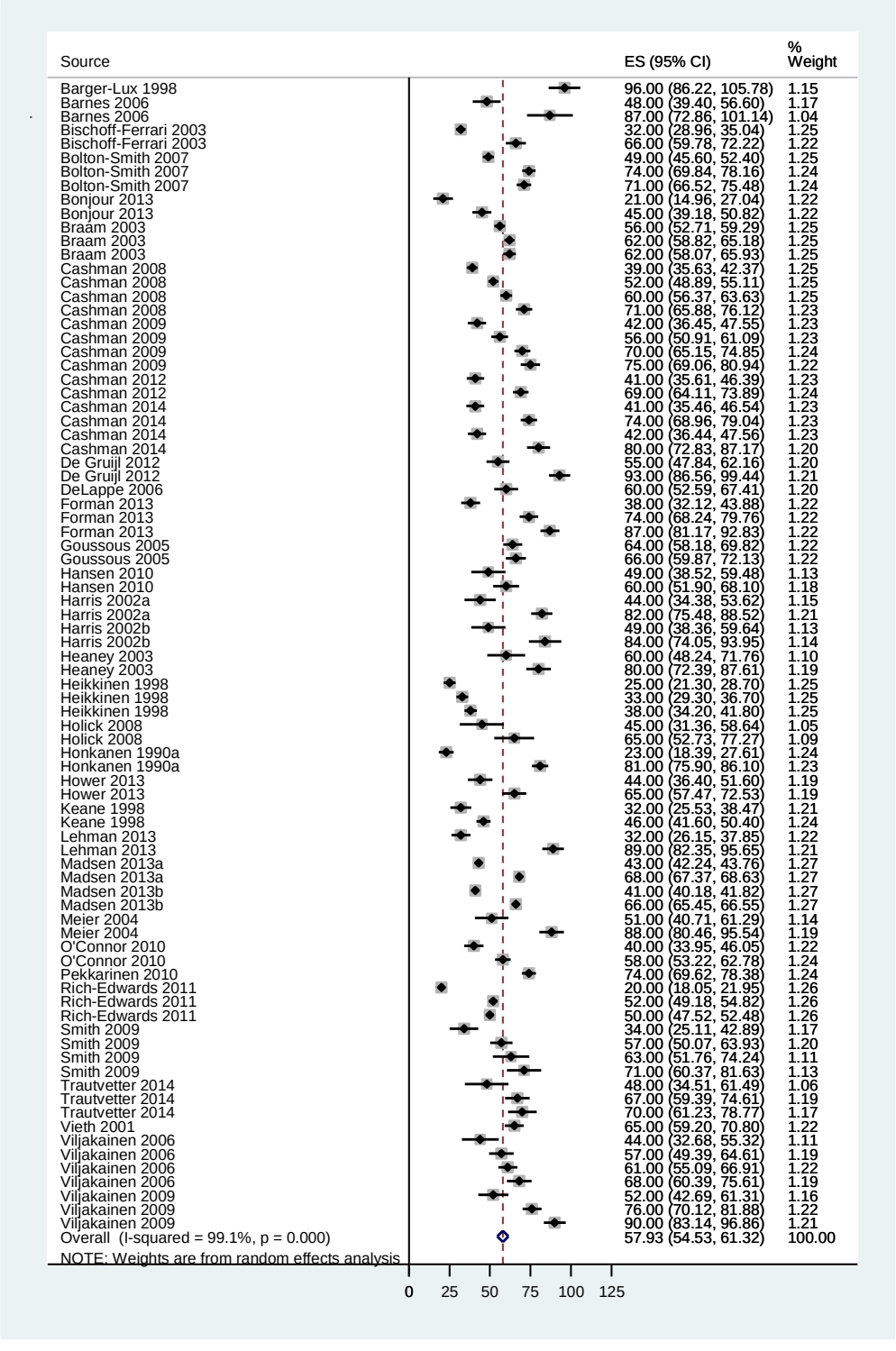
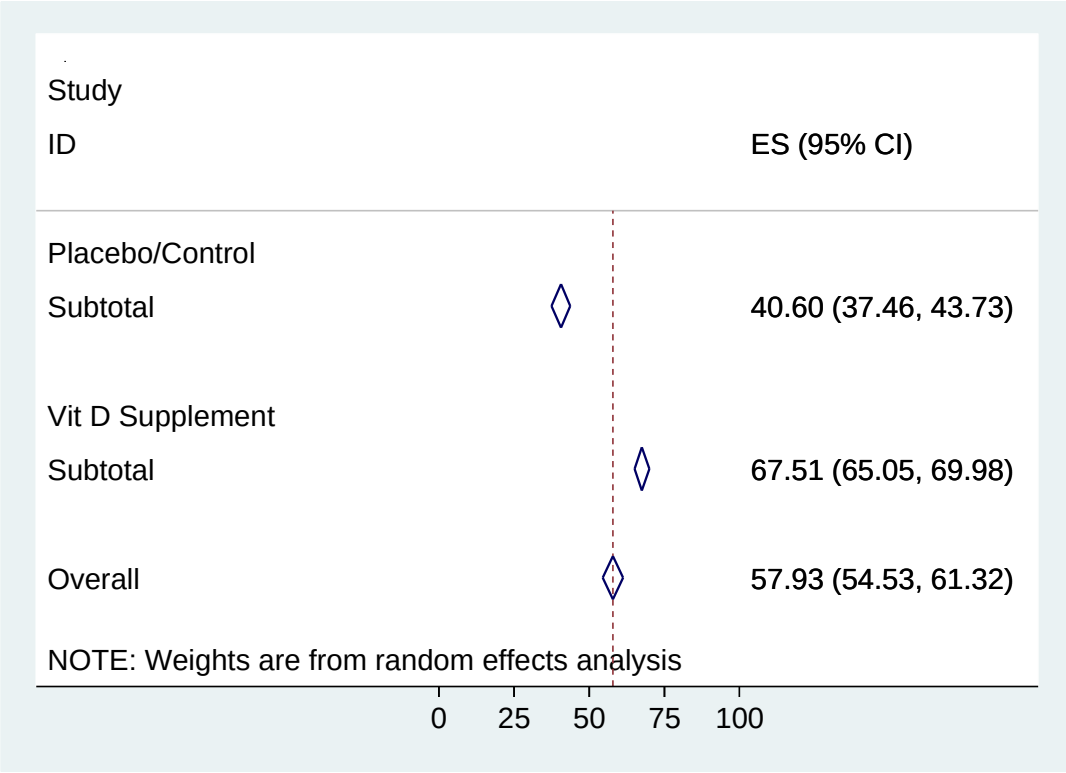


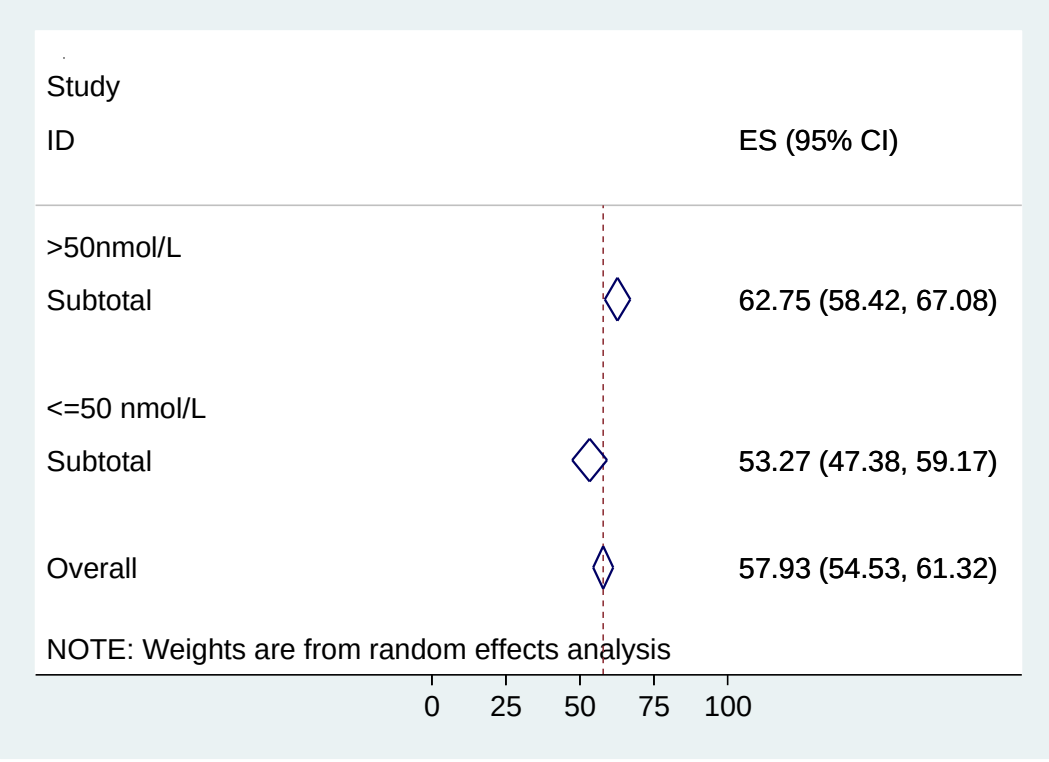
Figure 4: Achieved mean serum 25(OH)D (and 95% CI) by RCT and sorted by intervention arm (n = 83)

e.g. (Madsen et al., 2013)a and (Madsen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study, but different population groups (e.g. in this case: children and adults).



6343

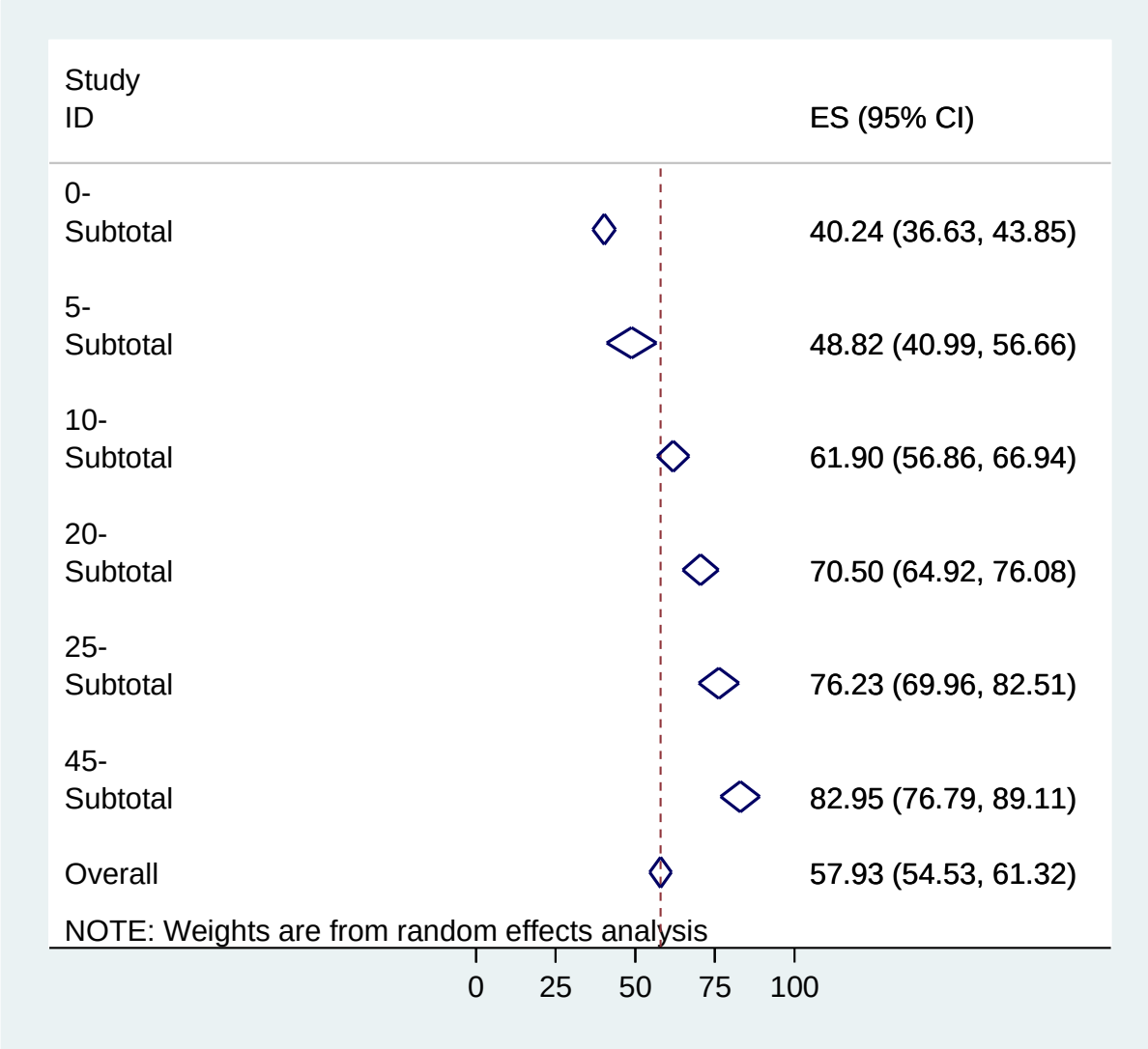
6344 **Figure 5:** Weighted pooled estimates of achieved mean serum 25(OH)D by INTERVENTION
6345 ARM



6346

6347 **Figure 6:** Weighted pooled estimates of achieved mean serum 25(OH)D by BASELINE MEAN
6348 serum 25(OH)D (nmol/L)

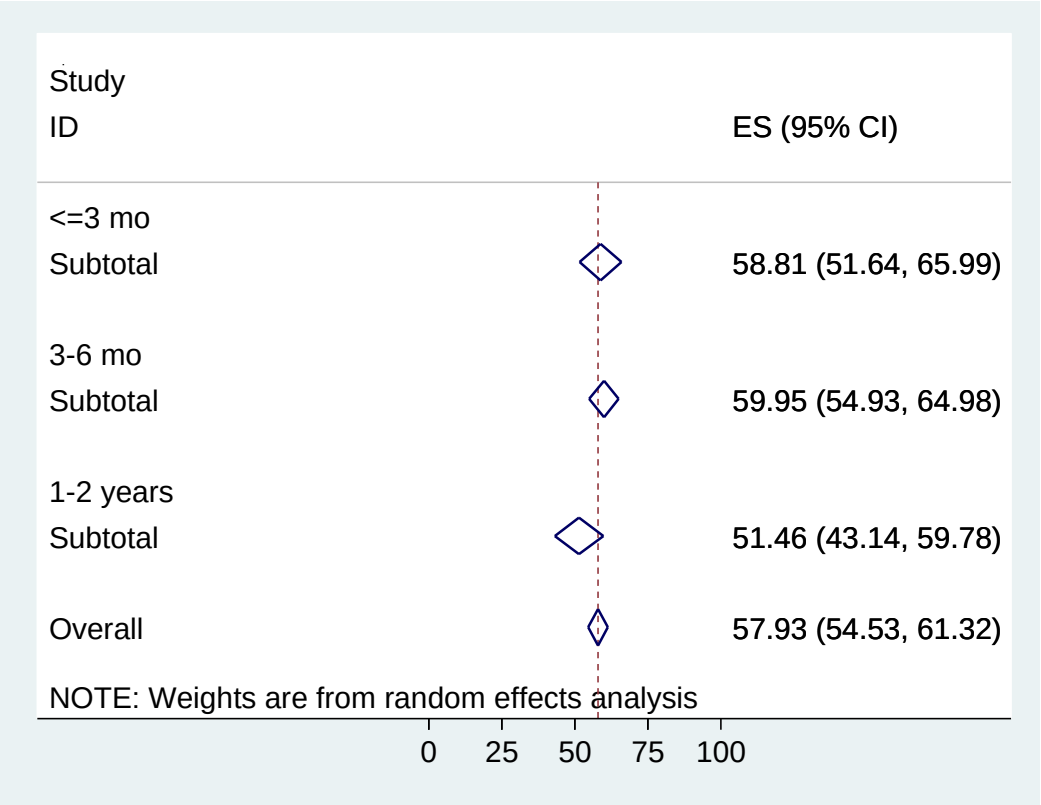
6349



6350

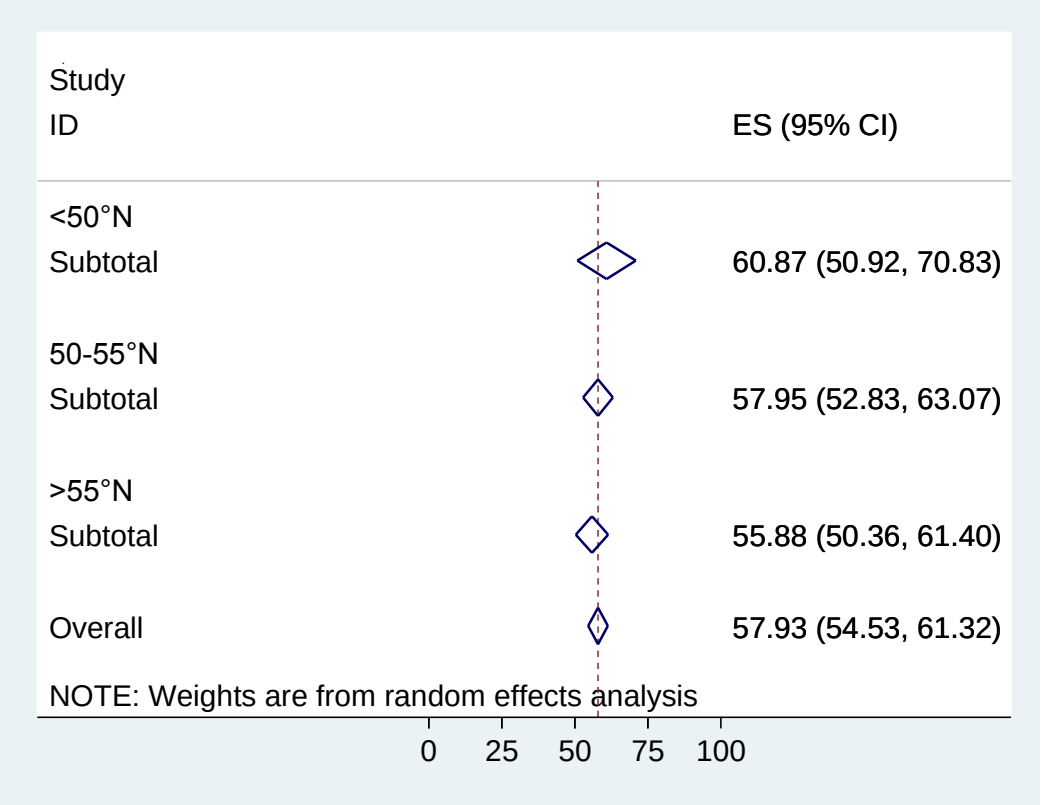
6351 **Figure 7:** Weighted pooled estimates of achieved mean serum 25(OH)D by TOTAL VITAMIN D
6352 INTAKE (µg/day)

6353



6354

6355 **Figure 8:** Weighted pooled estimates of achieved mean serum 25(OH)D by STUDY DURATION



6356

6357 **Figure 9:** Weighted pooled estimates of achieved mean serum 25(OH)D by LATITUDE

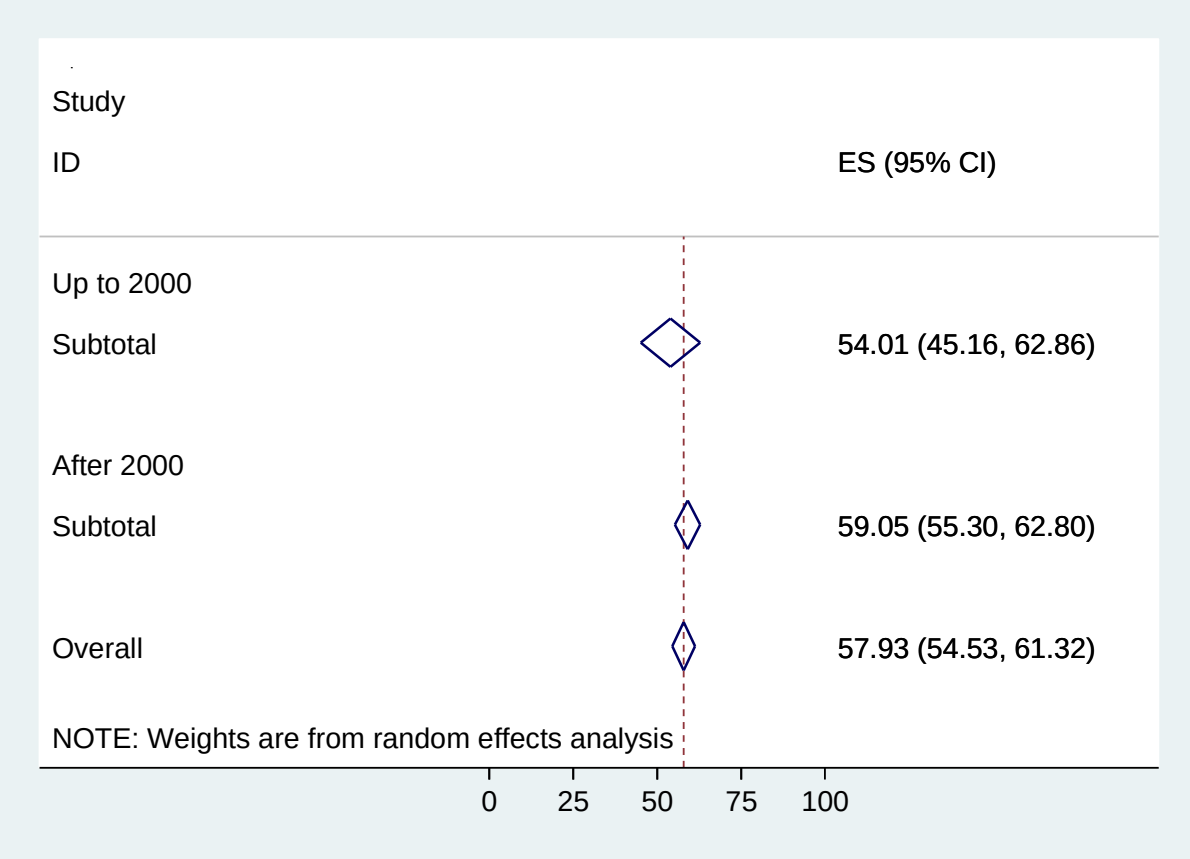
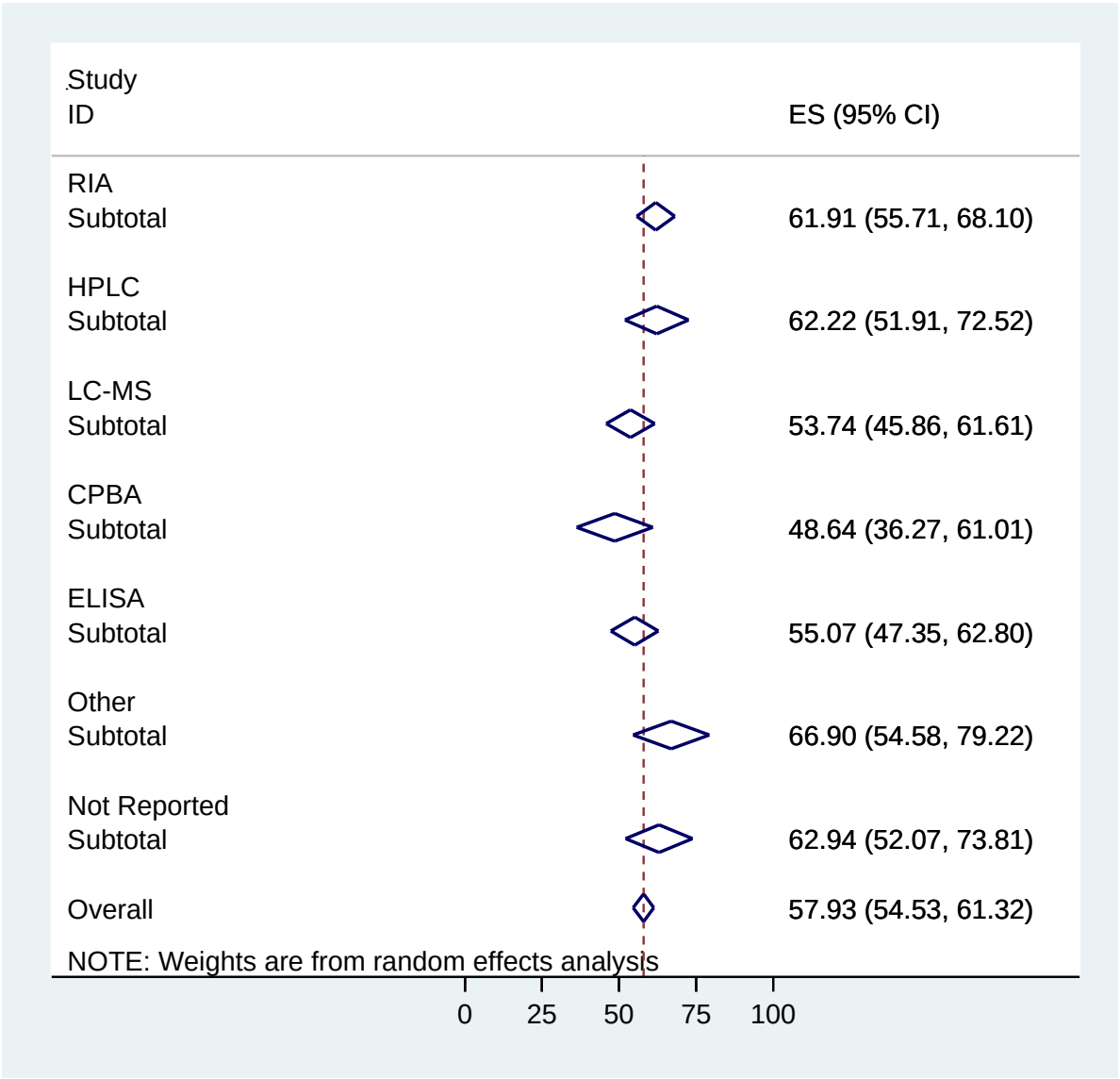


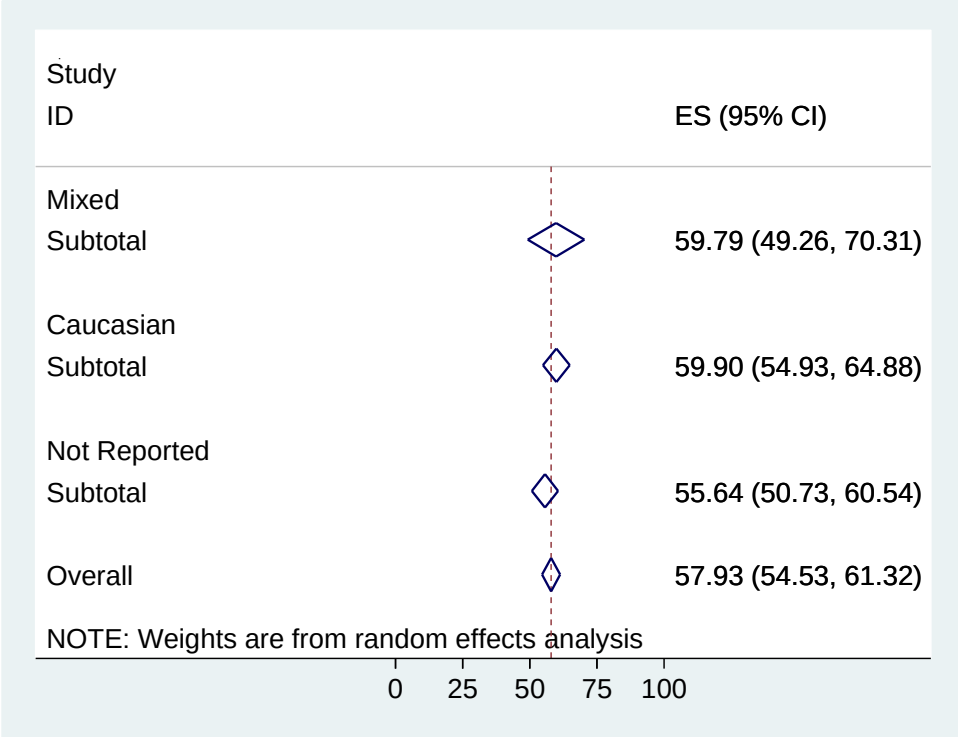
Figure 10: Weighted pooled estimates of achieved mean serum 25(OH)D by STUDY START PERIOD



6362

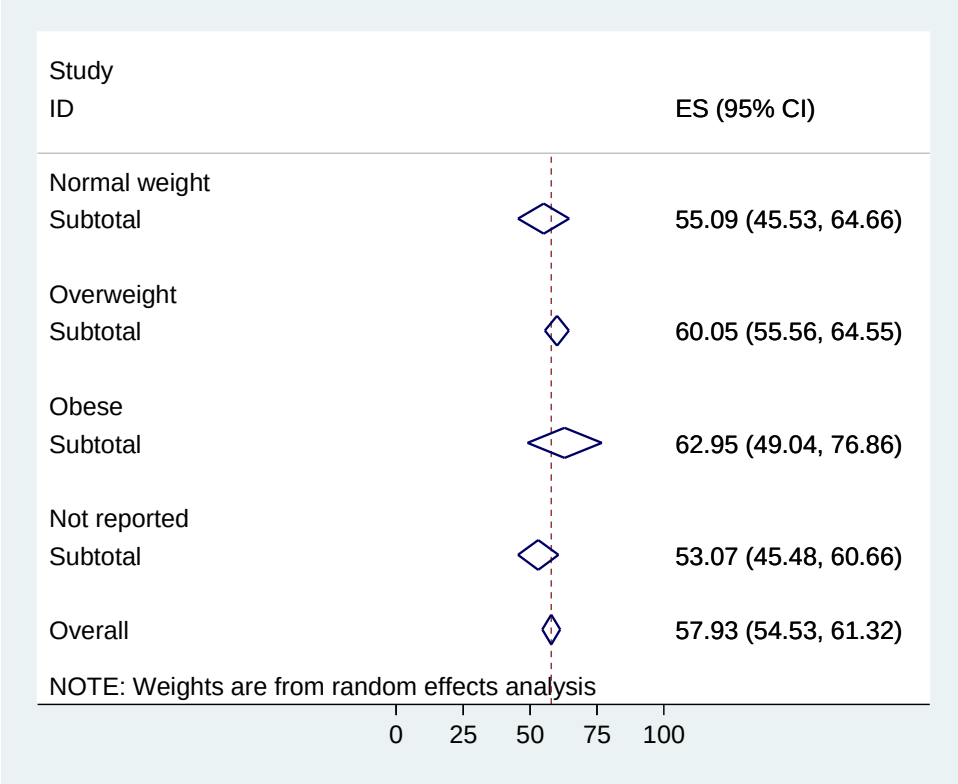
6363 **Figure 11:** Weighted pooled estimates of achieved mean serum 25(OH)D by ANALYTICAL
6364 METHOD

6365



6366

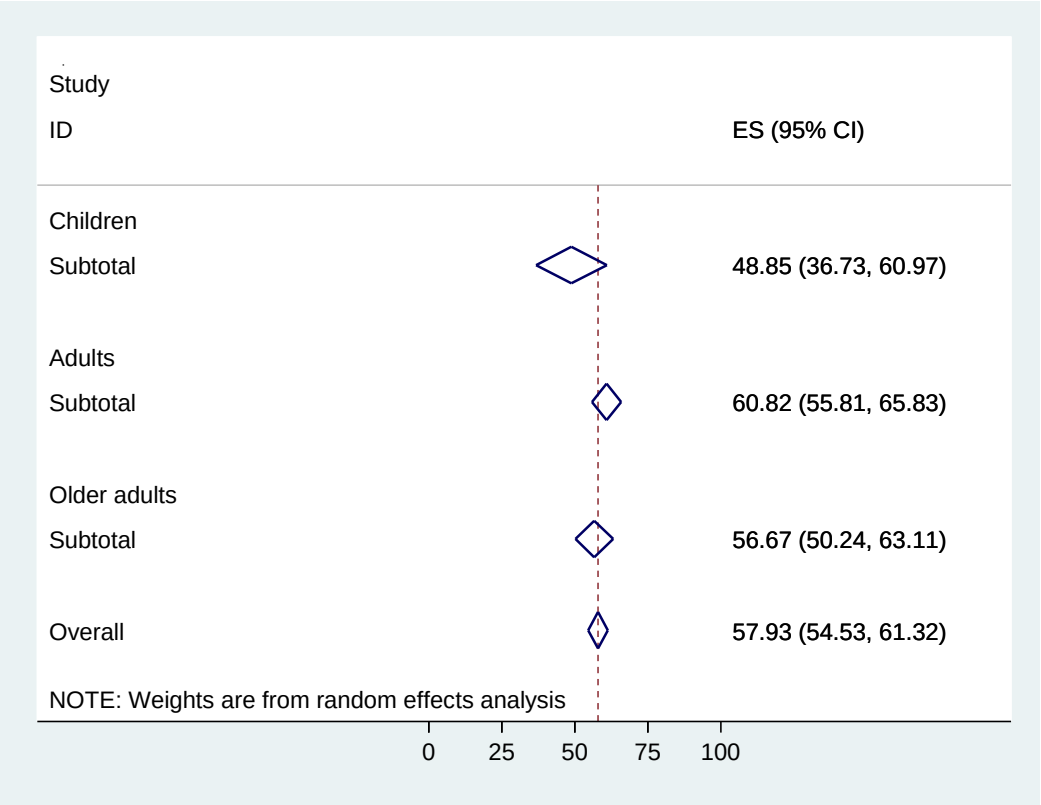
6367 **Figure 12:** Weighted pooled estimates of achieved mean serum 25(OH)D by ETHNICITY



6368

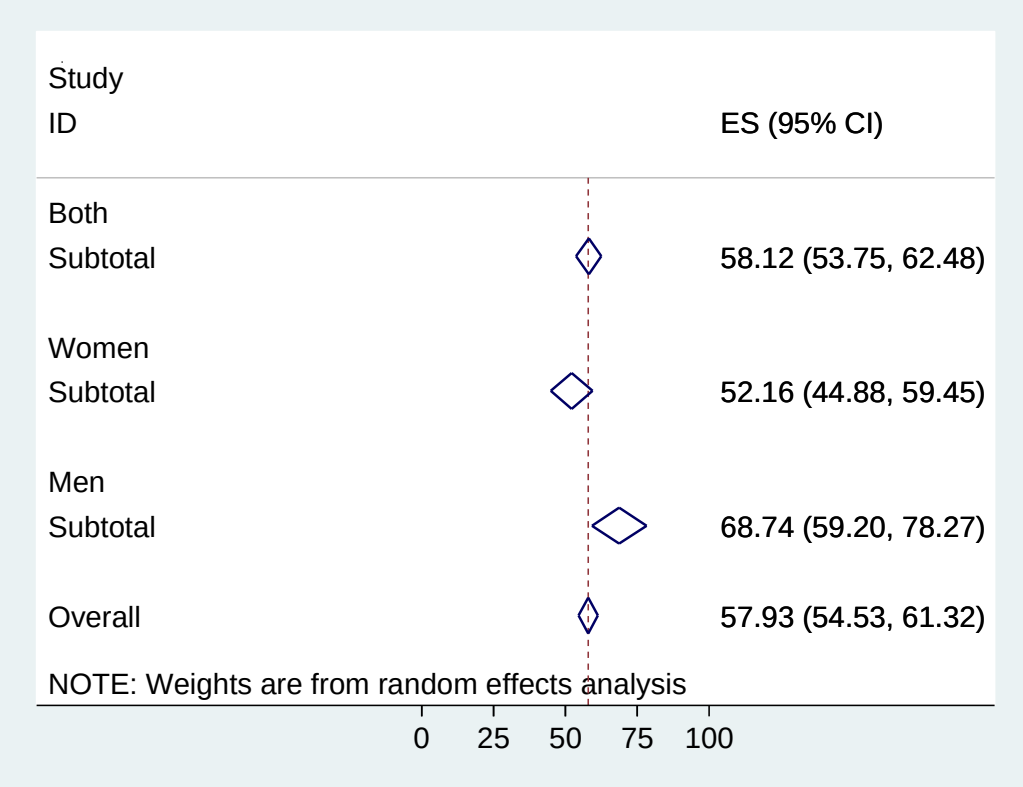
6369 **Figure 13:** Weighted pooled estimates of achieved mean serum 25(OH)D by mean BMI of the study
6370 population

6371 Normal weight: 18.5–24.9 kg/m^2, overweight: 25–29.9 kg/m^2, obese: 30 kg/m^2 and above.
6372 BMI, body mass index.



6373

6374 **Figure 14:** Weighted pooled estimates of achieved mean serum 25(OH)D by AGE



6375

6376 **Figure 15:** Weighted pooled estimates of achieved mean serum 25(OH)D by SEX

6377

D. *Forrest plots of mean differences in achieved serum 25(OH)D concentrations (intervention arm versus control arm) by relevant factors explored in the dose-response models*

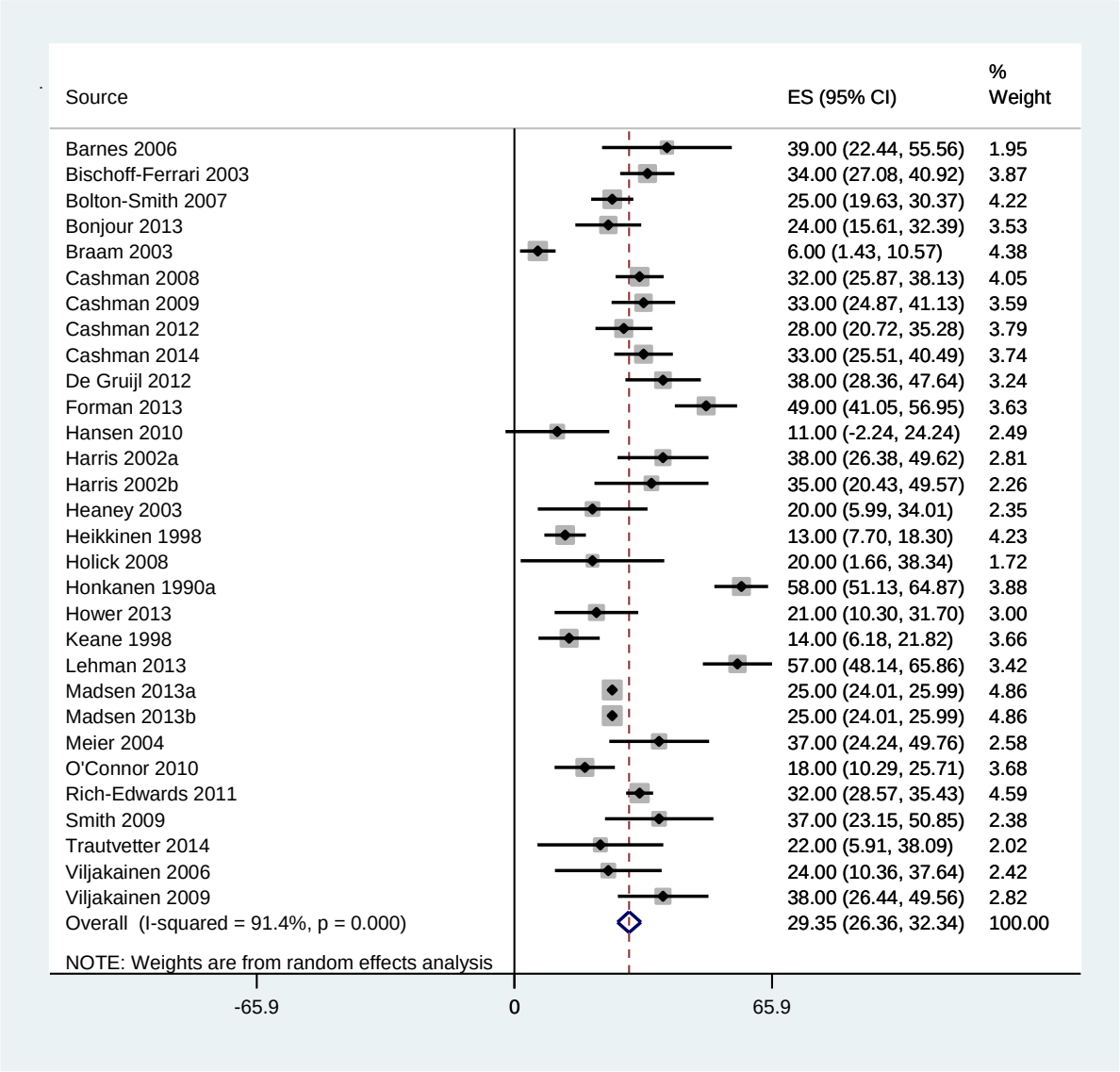
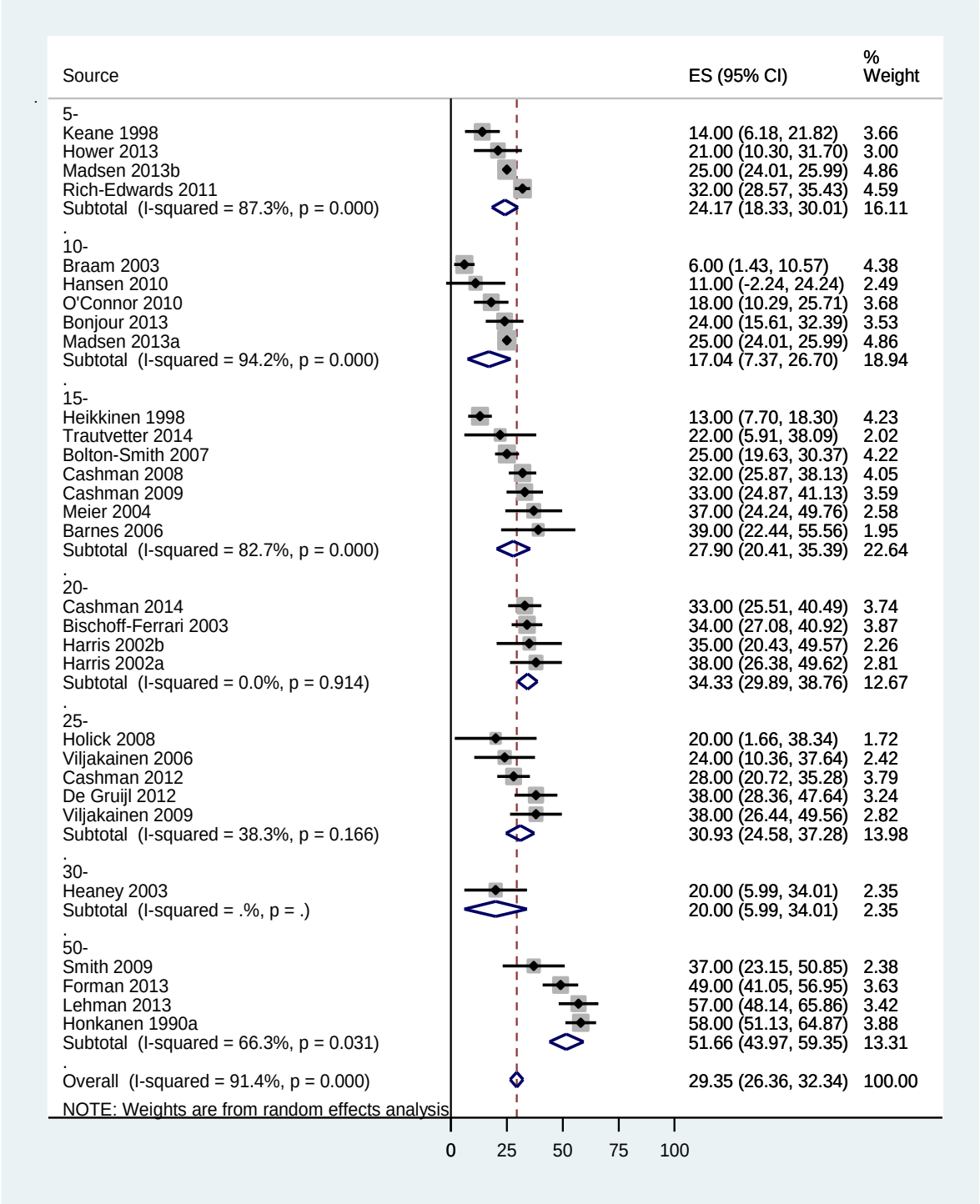


Figure 16: Mean differences in achieved serum 25(OH)D by RCT (n = 30) – random-effects meta-analysis

e.g. (Madsen et al., 2013)a and (Madsen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study, but different population groups (e.g. in this case: children and adults).



6387

6388 **Figure 17:** Weighted pooled estimates of mean differences in achieved serum 25(OH)D by TOTAL
6389 VITAMIN D INTAKE

6390

E. MODEL FITTING

Table 13: Regression coefficients from meta-regression models as covariates are fitted (first row: null model; second row: ln of total vitamin D intake; last row: fully adjusted model) and related Tau^2, Adjusted R^2 and residual I^2 value changes.

Ln of total vitamin D intake	Mean baseline 25(OH)D	Latitude	Start year	Assay (ELISA vs RIA)	Compliance assessed	Intercept	Tau^2	Adj R^2	I^2_{res}
						57.95***	**312**	**0**	**99%**
14.59* **						23.28***	137	56%	98%
15.15* **	0.531***					-4.98	69	78%	92%
15.74* **	0.507***	-0.478***				20.16**	55	82%	91%
15.93* **	0.481***	-0.460***	0.268			14.85	53	83%	90%
15.67* **	0.477***	-0.501***	0.598*	-6.308*		13.22	50	84%	88%
16.02* **	0.477***	-0.535***	0.783**	-6.300*	7.155*	9.23	**46**	**85%**	**87%**

* p < 0.05; ** p < 0.01; *** p < 0.001

Adj R^2, adjusted R^2; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay.

F. META-REGRESSION DOSE-RESPONSE MODELS; PREDICTED MEAN SERUM 25(OH)D, 95% CONFIDENCE INTERVAL AND 95% PREDICTION INTERVAL

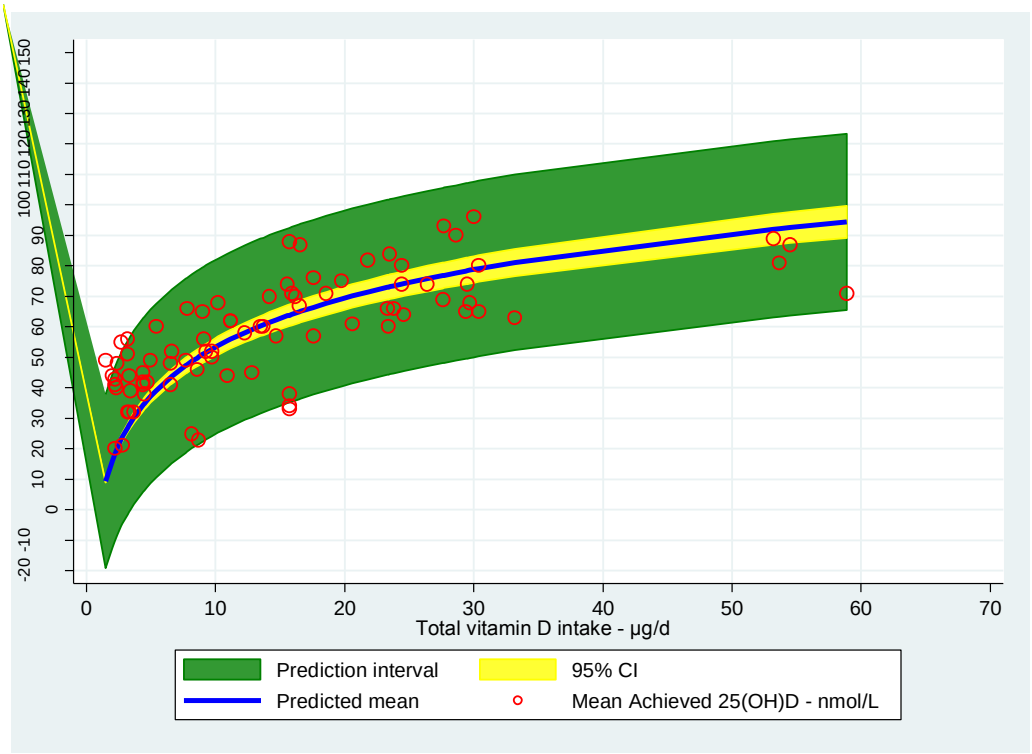


Figure 18: Meta-regression model of serum 25(OH)D response to ln of total vitamin D intake (unadjusted model) (n = 83)

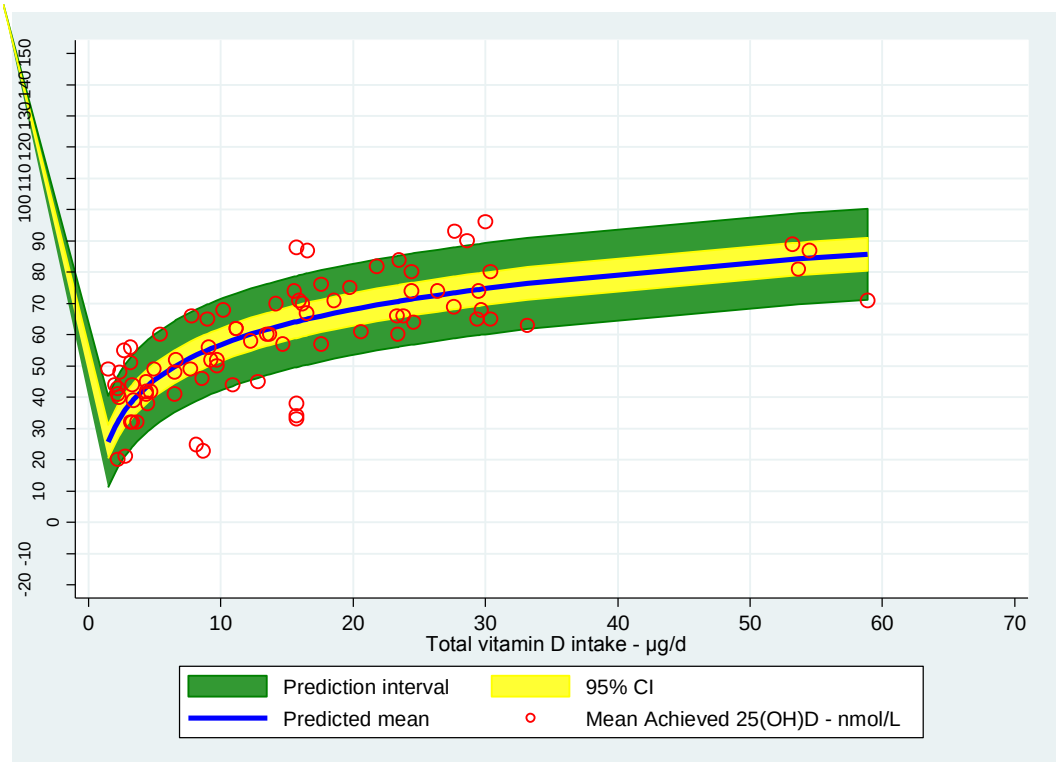


Figure 19: Meta-regression model of serum 25(OH)D response to ln of total vitamin D intake (adjusted model) (n = 83)

G. *Predicted achieved serum 25(OH)D and estimated total vitamin D intakes by age (adults, children) (74, 9 arms)*

Table 14: Predicted achieved serum 25(OH)D (nmol/L) at selected values of total vitamin D intake (µg/day) by AGE

Regression equations used to predict serum 25(OH)D	ADULTS (74 arms)						CHILDREN (9 arms)					
	Predicted serum 25(OH)D (nmol/L) at selected values of total vitamin D intake (µg/day)						Predicted serum 25(OH)D (nmol/L) at selected values of total vitamin D intake (µg/day)					
	100	50	20	15	10	5	100	50	20	15	10	5
Unadjusted models	y = ln (total vitamin D intake) §						y = ln (total vitamin D intake) §					
Predicted mean	106	90	69	62	53	37	124	106	81	73	62	43
95% CI lower limit	100	85	65	59	50	35	94	80	61	56	47	33
95% CI upper limit	112	95	73	66	56	39	154	131	100	91	77	54
95% PI lower limit	77	61	40	34	24	9	82	65	42	35	24	7
95% PI upper limit	134	118	97	91	81	65	166	146	120	112	100	80
Adjusted models ł	y = ln (total vitamin D intake) + mean baseline 25(OH)D + latitude + start year + HPLC + LC-MS + CPBA + ELISA/nr + Other assay + Compliance not ass. §						y = ln (total vitamin D intake) + mean baseline 25(OH)D §					
Predicted mean	95	83	68	63	56	45	*101*	*88*	*72*	*67*	*60*	*47*
95% CI lower limit	89	77	62	57	51	39	*93*	*81*	*66*	*61*	*54*	*42*
95% CI upper limit	100	89	74	69	62	51	*108*	*95*	*78*	*73*	*65*	*53*
95% PI lower limit	80	68	53	48	41	30	*89*	*77*	*61*	*55*	*48*	*36*
95% PI upper limit	110	98	83	78	71	60	*113*	*100*	*84*	*78*	*71*	*59*

CI, Confidence interval; PI, Prediction interval.
§ General predictive regression equations are reported.
ł **Estimates from the adjusted models are based on all covariates set to their mean values.**

6414 **Table 15:** Estimated vitamin D intakes (µg/day) at selected serum 25(OH)D cut-off values (nmol/L) by AGE

Regression equations used to estimate vitamin D intake	ADULTS (74 arms) Estimated vitamin D intake at selected serum 25(OH)D cut-off values (nmol/L)				CHILDREN (9 arms) Estimated vitamin D intake at selected serum 25(OH)D cut-off values (nmol/L)			
	50	40	30	25	50	40	30	25
Unadjusted models	y = ln (total vitamin D intake) §				y = ln (total vitamin D intake) §			
Predicted mean	8.8	5.7	3.7	3.0	6.4	4.4	3.0	2.5
95% CI lower limit	10.1	6.3	4.0	3.2	11.5	7.0	4.3	3.4
95% CI upper limit	7.9	5.2	3.4	2.8	4.4	3.3	2.4	2.1
95% PI lower limit	30.6	19.7	12.7	10.2	27.6	18.5	12.5	10.2
95% PI upper limit	2.6	1.7	1.1	0.9	1.8	1.3	0.9	0.7
Adjusted models ł	y = ln (total vitamin D intake) + mean baseline 25(OH)D + latitude + start year + HPLC + LC-MS + CPBA + ELISA/nr + Other assay + Compliance not ass. §				y = ln (total vitamin D intake) + mean baseline 25(OH)D §			
Predicted mean	6.8	3.7	2.0	1.5	*5.8*	*3.3*	*1.9*	*1.4*
95% CI lower limit	9.6	5.2	2.9	2.1	*7.9*	*4.4*	*2.4*	*1.8*
95% CI upper limit	4.8	2.6	1.4	1.1	*4.3*	*2.5*	*1.5*	*1.1*
95% PI lower limit	16.9	9.2	5.0	3.7	*10.9*	*6.2*	*3.5*	*2.6*
95% PI upper limit	2.7	1.5	0.8	0.6	*3.1*	*1.8*	*1.0*	*0.8*

6415
6416 CI, confidence interval; PI, prediction interval.
6417 § General predictive regression equations are reported.
6418 ł **Estimates from the adjusted models are based on all covariates set to their mean values.**

H. SENSITIVITY ANALYSES

Table 16: Adjusted meta-regression models on subsets of the final data set after exclusions of trials with specific characteristics

Adjusted Ln of Total vitamin D intake - µg/day (covariates coefficients not reported)	Coefficient	95% CI		Number of observations	Residual I-squared
FINAL MODEL	16.3	*14.5*	*18.2*	83	87%
Models restricted to trials without:					
Recruitment of patient groups	16.4	*14.4*	*18.4*	78	87%
Vitamin D supplement users	16.8	*14.5*	*19.1*	52	86%
Persons with sun holiday during trial	18.0	*14.9*	*21.2*	41	85%
Persons using sunbeds/artificial UV-B	16.5	*13.3*	*19.8*	31	78%
Users of medication	16.0	*13.8*	*18.1*	42	85%
Participants with diseases known to interfere with vitamin D metabolism	17.5	*15.3*	*19.8*	43	84%

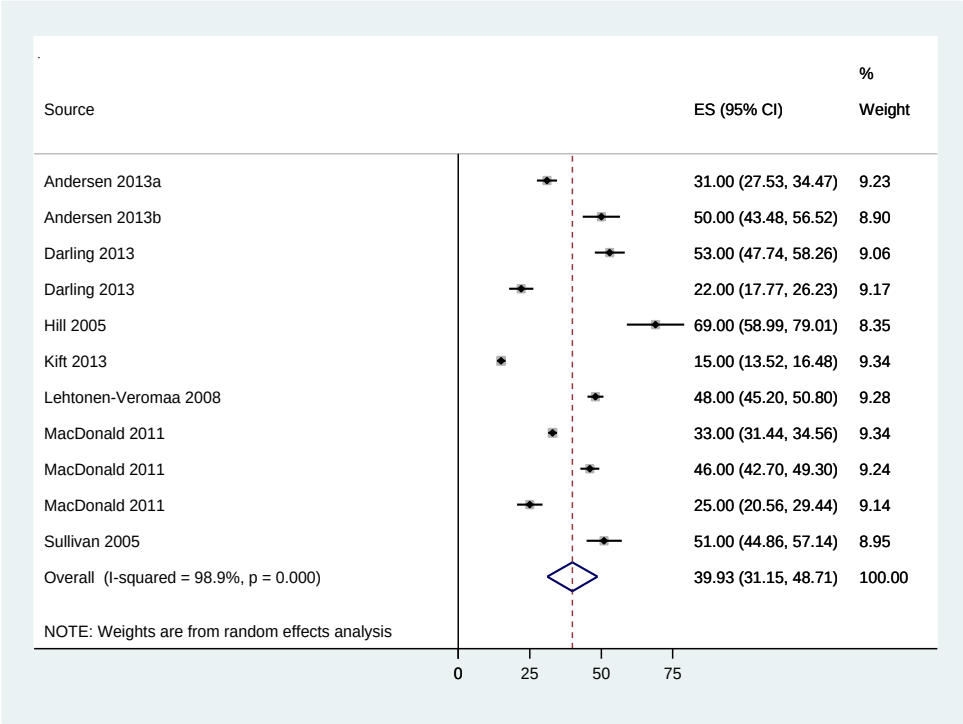
6426 *I. PROSPECTIVE OBSERVATIONAL STUDIES*
6427
6428 **Table 17:** Prospective observational studies – main study characteristics

Source	Publication year	Country	Latitude	Age Mean	Male %	Ethnicity	Duration
(Andersen et al., 2013)a	2013	DK	55.4	13	0	-	52
(Andersen et al., 2013)b	2013	DK	55.4	72	0	-	52
(Darling et al., 2013)	2013	UK	51	34	0	Mixed	13
(Darling et al., 2013)	2013	UK	51	38	0	Mixed	13
(Hill et al., 2005)	2005	IE	51	60	0	-	52
(Kift et al., 2013)	2013	UK	53.5	24	67	Asian	13
(Lehtonen-Veromaa et al., 2008)	2008	FI	60.3	16	0	Caucasian	208
(MacDonald et al., 2011)	2011	UK	57	62	0	Mixed	65
(MacDonald et al., 2011)	2011	UK	57	62	0	Mixed	65
(MacDonald et al., 2011)	2011	UK	57	61	0	Mixed	65
(Sullivan et al., 2005)	2005	USA	44	11	0	-	104

6429

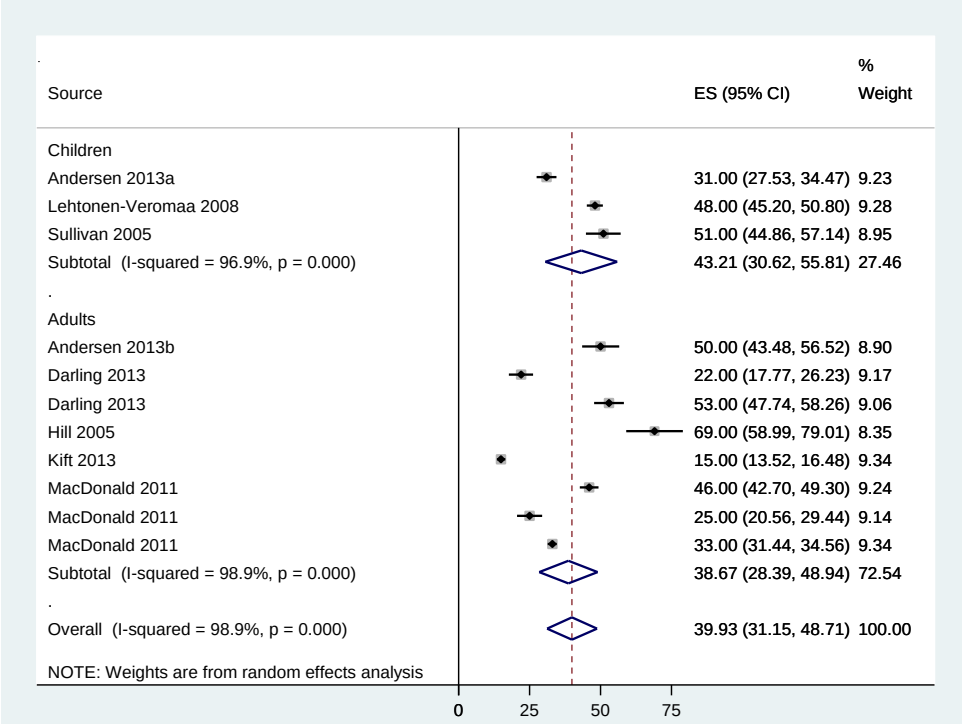
Source	Total vitamin D intake	Participants per group	Baseline Mean 25(OH)D	Baseline 25(OH)D SD	Achieved Mean 25(OH)D	Achieved 25(OH)D SD
(Andersen et al., 2013)a	3.9	54	23	14	30	13
(Andersen et al., 2013)b	8.1	52	47	25	51	24
(Darling et al., 2013)	2.6	80	45	18	53	24
(Darling et al., 2013)	2.0	26	20	11	22	11
(Hill et al., 2005)	5.8	47	55	28	69	35
(Kift et al., 2013)	1.4	86	20	7	15	7
(Lehtonen-Veromaa et al., 2008)	4.0	142	48	20	48	17
(MacDonald et al., 2011)	3.6	308	32	14	33	14
(MacDonald et al., 2011)	3.1	114	44	18	46	18
(MacDonald et al., 2011)	2.0	28	24	12	25	12
(Sullivan et al., 2005)	5.4	20	56	17	51	14

6430 DK, Denmark; FI, Finland; IE, Ireland; SD, standard deviation; UK, United Kingdom, USA; United States of America.
6431 e.g. (Andersen et al., 2013)a and (Andersen et al., 2013)b (as cited in Brouwer-Brolsma et al. (2016)) refer to the same study,
6432 but different population groups (e.g. in this case: children and adults).
6433



6434

6435 **Figure 20:** Achieved mean serum 25(OH)D (and 95% CI) by study group (n = 11)



6436

6437 **Figure 21:** Achieved mean serum 25(OH)D (and 95% CI) by age group

6438

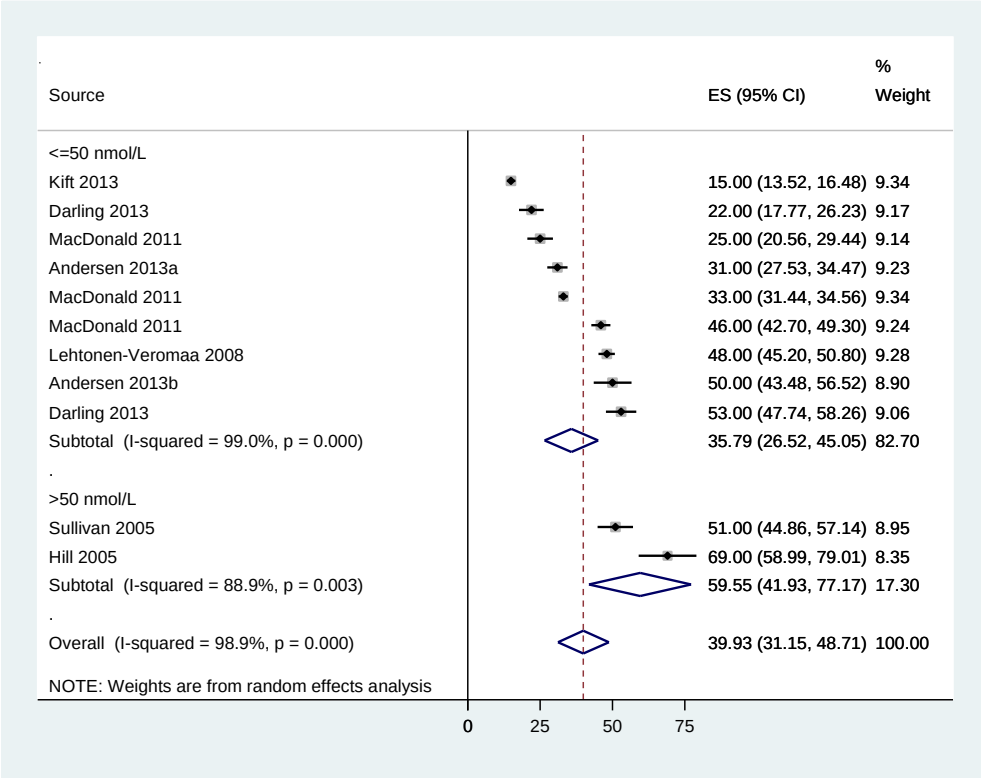


Figure 22: Achieved mean serum 25(OH)D (and 95% CI) by baseline mean serum 25(OH)D

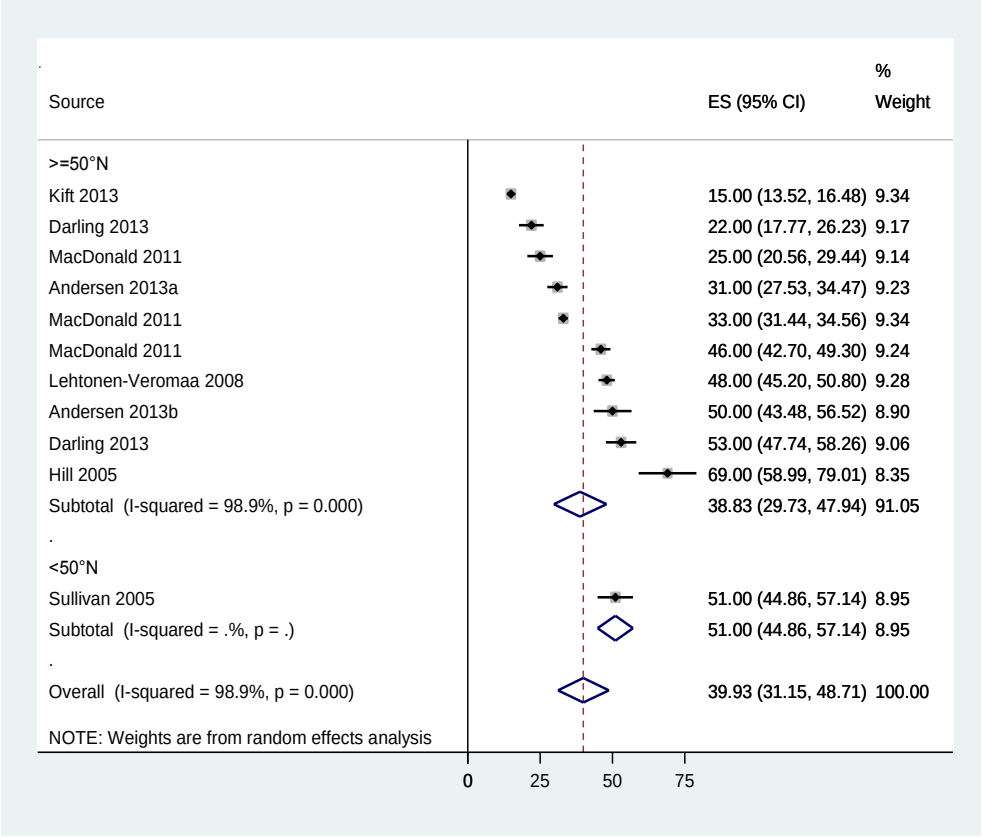
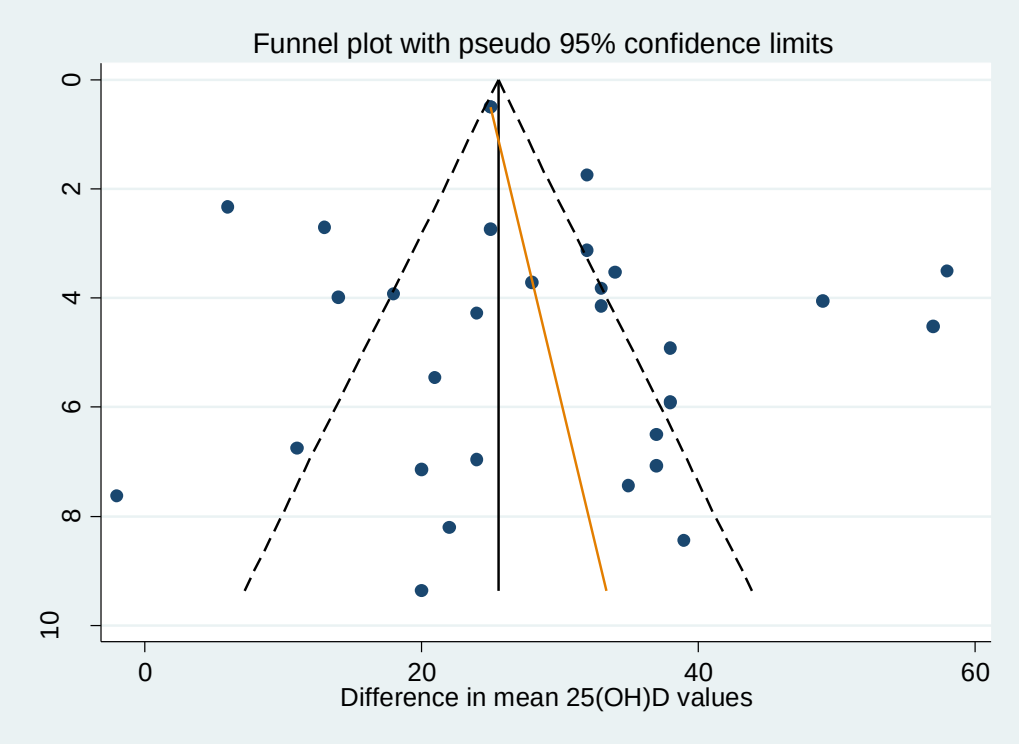


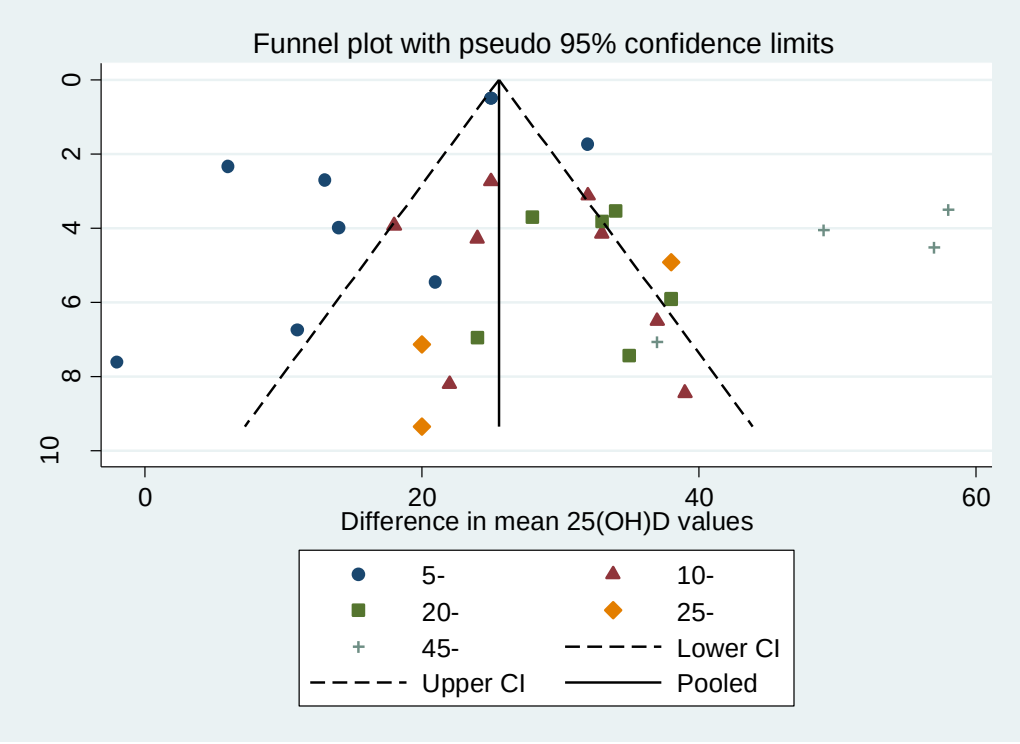
Figure 23: Achieved mean serum 25(OH)D (and 95% CI) by latitude

6444 J. FUNNELS PLOTS OF MEAN DIFFERENCES IN ACHIEVED SERUM 25(OH)D FROM 30 RCTS (STUDIES INCLUDED IN
6445 THE META-ANALYSES) AND EGGER'S TEST FOR SMALL-STUDY EFFECTS.



6446

6447 **Figure 24:** Funnel plot of mean differences and Egger's regression line



6448

6449 **Figure 25:** Funnel plot of mean differences by vitamin D dose categories

6450 CI, confidence interval; SE of MD: standard error of mean difference.
6451

6452 **ABBREVIATIONS**

$1,25(OH)_2D$	1,25-dihydroxy-vitamin D
$1,25(OH)_2D_2$	1,25-dihydroxy-ergocalciferol
$1,25(OH)_2D_3$	1,25-dihydroxy-cholecalciferol
$1,24,25(OH)_3D$	1,24,25-trihydroxyvitamin D
25(OH)D	25-hydroxy-vitamin D (sum of 25-hydroxy-vitamin D_2 and 25-hydroxy-vitamin D_3)
7-DHC	7-dehydrocholesterol
aBMD	Areal bone mineral density
ADL	Activities of daily living
Afssa	Agence française de sécurité sanitaire des aliments
AHRQ	Agency for Healthcare Research and Quality
AI	Adequate Intake
ALP	Alkaline phosphatase
AMD	Age-related macular degeneration
AR	Average Requirement
BA	Bone area
BioE	Bioavailable estradiol
BioT	Bioavailable testosterone
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BV	Bone volume
CI	Confidence interval
CPBA	Competitive protein binding assay
CSA	Cross-sectional area
CVD	Cardiovascular disease
CYP	Cytochrome P450

CYP24A1	24-hydroxylase
CYP27B1	1α-hydroxylase
CYP2R1, CYP27A1, CYP3A4, CYP2J3	25-hydroxylase
D-A-CH	Deutschland- Austria- Confoederatio Helvetica
DEQAS	Vitamin D External Quality Assessment Scheme
DBP	Vitamin D-binding protein
DHCR7	7-dehydrocholesterol reductase
DH	UK Department of Health
DRV	Dietary Reference Values
DXA	Dual-energy X-ray absorptiometry
EAR	Estimated Average Requirement
EC	European Commission
ELISA	Enzyme-linked immunosorbent assay
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
EU	European Union
FAO	Food and Agriculture Organisation
FGF-23	Fibroblast growth factor 23
GC	Group specific component gene
GWAS	Genome-wide association studies
HPLC	High-performance liquid chromatography
HR	Hazard ratio
I^2	Heterogeneity index
IOM	U.S. Institute of Medicine of the National Academy of Sciences
IQR	Interquartile range
ITT	Intention-to-treat
IU	International unit

LC-MS	Liquid chromatography-mass spectroscopy
LC-MS/MS	Liquid chromatography-tandem mass spectroscopy
LMQ	Leg muscle quality
NCM	Nordic Council of Ministers
NHANES	United States National Health and Nutrition Examination Survey
NIST	National Institute of Standards and Technology
NNR	Nordic Nutrition Recommendations
NOAEL	No Observed Adverse Effect Level
OR	Odds ratio
PI	Prediction interval
pQCT	Peripheral quantitative computed tomography
PRI	Population reference intake
Q1	First quartile
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RDA	Recommended Dietary Allowance
PTH	Parathyroid hormone
RCT	Randomised controlled trial
RI	Recommended Intake
RIA	Radioimmunoassay
RMP	Reference measurement procedure
RNI	Reference Nutrient Intake
RoB	Risk of bias
RR	Relative risk
SACN	Scientific Advisory Committee on Nutrition
SGA	Small-for-gestational-age
SCF	Scientific Committee for Food

SD	Standard deviation
SH	Sex hormones
SHBG	Sex hormone binding globulin
SPPB	Short physical performance battery
SSI	Stress-strain index
TUAG	Timed Up And Go
UHT	Ultra-high temperature
UK	United Kingdom
UL	Tolerable Upper Intake Level
UV	Ultraviolet
vBMD	Volumetric bone mineral density
VDR	Vitamin D receptor
VDSP	Vitamin D standardization program
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
WHO	World Health Organization

6453