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Scientific opinion on the tolerable upper intake level for vitamin D, including the derivation of a conversion factor for calcidiol monohydrate

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Abstract

Following two requests from the European Commission (EC), the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver a scientific opinion on the revision of the tolerable upper intake level (UL) for vitamin D and to propose a conversion factor (CF) for calcidiol monohydrate into vitamin D_3 for labelling purposes. Vitamin D refers to ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3), and calcidiol monohydrate. Systematic reviews of the literature were conducted to assess the relative bioavailability of calcidiol monohydrate versus vitamin D_3 on serum 25(OH)D concentrations, and for priority adverse health effects of excess vitamin D intake, namely persistent hypercalcaemia/hypercalciuria and endpoints related to musculoskeletal health (i.e. falls, bone fractures, bone mass/density and indices thereof). Based on the available evidence, the Panel proposes a CF for calcidiol monohydrates of 2.5 for labelling purposes. Persistent hypercalciuria, which may be an earlier sign of excess vitamin D than persistent hypercalcaemia, is selected as the critical endpoint on which to base the UL for vitamin D. A lowest-observed-adverse-effect-level (LOAEL) of 250 μg/day is identified from two randomised controlled trials in humans, to which an uncertainty factor of 2.5 is applied to account for the absence of a no-observed-adverse-effect-level (NOAEL). A UL of 100 μg vitamin D equivalents (VDE)/day is established for adults (including pregnant and lactating women) and for adolescents aged 11–17 years, as there is no reason to believe that adolescents in the phase of rapid bone formation and growth have a lower tolerance for vitamin D compared to adults. For children aged 1–10 years, a UL of 50 μg VDE/day is established by considering their smaller body size. Based on available intake data, European populations are unlikely to exceed the UL, except for regular users of food supplements containing high doses of vitamin D.

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1. Introduction

Directive 2002/46/EC[1] on food supplements and Regulation (EC) No 1925/2006[2] on fortified foods delegate the power to the European Commission (EC) to adopt maximum amounts of vitamins and minerals that may be used in food supplements or added to foods. In this context, the EC asked EFSA to update the scientific advice on the Tolerable Upper Intake Level (UL) for a number of priority nutrients, among which vitamin D.[3]

On 25 February 2022, during the development of the protocol to update the UL for vitamin D (Annex A), the Commission asked EFSA to assess the extent to which calcidiol monohydrate (i.e., 25-hydroxycholecalciferol monohydrate) is bioavailable as compared to native vitamin D_3, and to derive a conversion factor that allows to convert absolute amounts of this nutrient form in μg into μg of native vitamin D_3. This question has been incorporated into the protocol and is addressed in this opinion (see Sections 1.1.2 and 1.5).

1.1. Background as provided by the European Commission

1.1.1. Tolerable upper intake level for vitamin D

Article 6 of Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods and Article 5 of Directive 2002/46/EC on the approximation of the laws of the Member States relating to food supplements provide that maximum amount of vitamins and minerals added to foods and to food supplements respectively, shall be set.

The above-mentioned provisions lay down the criteria to be taken into account when establishing these maximum amounts that include the upper safe levels (ULs) of vitamins and minerals established by scientific risk assessment based on "generally accepted scientific data, taking into account, as appropriate, the varying degrees of sensitivity of different groups of consumers".

To set maximum amounts of vitamins and minerals in fortified foods and food supplements, the Commission would like to ask the European Food Safety Authority (EFSA) to review the previous opinions of the Scientific Committee on Food (SCF) or the NDA Panel on the ULs for vitamin A,[4] folic acid/folate,[4] vitamin D,[4] vitamin E,[4] vitamin B6, iron,[4] manganese[4] and β-carotene[4] to take into account recent scientific developments and evidence.

In this context, EFSA should first review the guidelines of the SCF[4] for the development of tolerable upper intake levels for vitamins and minerals (adopted on 19 October 2000).

Tolerable Upper Intake Levels should be presented separately for the age group from 4/6 months onwards until 3 years of age and the general population group from 3 years onwards, taking into account, as appropriate, the varying degrees of sensitivity of different consumer groups. As foods intended for the general population are also consumed by young children, young children should be considered as a potentially sensitive consumer group.

1.1.2. Conversion factor for calcidiol monohydrate

Annex II to Directive 2002/46/EC lists the chemical substances that may be used as forms of vitamins and minerals in the manufacture of food supplements.

Following a request from the Commission, EFSA adopted a Scientific Opinion on the safety of calcidiol monohydrate (25-hydroxycholecalciferol monohydrate) as a novel food pursuant to Regulation (EU) 2015/2283[5], including its bioavailability as a metabolite of vitamin D_3 when added for nutritional purposes to food supplements (EFSA NDA Panel, 2021a).

[1] 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. 51–57.

[2] 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. 26–38.

[3] EFSA Mandate No M-2021-00058 of 7 June 2021.

[4] SCF (2000). Scientific Committee on Food. Guidelines of the Scientific Committee on Food for the Development of Tolerable Upper Intake Levels for Vitamins and Minerals. in: Scientific Committee on Food, Scientific Panel on Dietetic Products, Nutrition and Allergies (2006). Tolerable Upper Intake Levels for Vitamins and Minerals. European Food Safety Authority. SCF (2001). Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Magnesium. in: Scientific Committee on Food, Scientific Panel on Dietetic Products, Nutrition and Allergies (2006). Tolerable Upper Intake Levels for Vitamins and Minerals. European Food Safety Authority.

[5] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods. OJ L 327, 11.12.2015, p. 1–22.

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In its Scientific Opinion, EFSA concluded that calcidiol monohydrate is safe under the proposed conditions of use and use levels for individuals ≥ 11 years old, including pregnant and lactating women and that it is a bioavailable source of the biologically active metabolite of vitamin D, i.e. 1,25-dihydroxyvitamin D. It was further noted that "a systematic review of data, assessing the extent to which oral calcidiol is more bioavailable than oral vitamin D_3 in all population groups and dietary context was outside the remit of this opinion and the data provided by the applicant do not permit this question to be answered for the proposed daily intake of 5 or 10 μg/day. Thus, as a theoretical calculation for this opinion, the NDA Panel used the factor of 5 set by the FEEDAP Panel to convert calcidiol to vitamin D."

Article 6(3) of Directive 2002/46/EC provides that the amount of the nutrients or substances with a nutritional or physiological effect present in the product shall be declared on the labelling in numerical form. Concerns have been raised by Member States that the absence of a conversion factor that would allow to convert the amount of calcidiol monohydrate into vitamin D_3 might cause difficulties for the national competent authorities in enforcing compliance with the abovementioned provision.

In addition, both Regulation (EU) No 1169/2011[6] and Directive 2002/46/EC foresee that the information on vitamins and minerals in a product shall be expressed as a percentage of the daily reference intakes. Annex XIII of Regulation (EU) No 1169/2011 lists these daily reference intakes, including that for vitamin D, without providing for a conversion factor that would allow to convert the amount of calcidiol monohydrate into vitamin D.

1.2. Terms of reference as provided by the European Commission

1.2.1. Tolerable upper intake level for vitamin D

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to:

1) Update the guidelines of the SCF for the development of Tolerable Upper Intake Levels for vitamins and minerals in the light of available recent scientific and methodological developments.
2) Review existing scientific evidence and provide advice on Tolerable Upper Intake Levels for the following vitamins and minerals including their currently authorised forms for the addition to fortified foods and food supplements for the general population and, as appropriate, for vulnerable subgroups of the population:

- vitamin A.
- folic acid/folate.
- vitamin D.
- vitamin E.
- iron.
- manganese.
- β-carotene.
- vitamin B6.

For nutrients for which there are no, or insufficient, data on which to base the establishment of an UL, an indication should be given on the highest level of intake where there is reasonable confidence in data on the absence of adverse effects.

1.2.2. Conversion factor for calcidiol monohydrate

In accordance with Article 29 of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to assess the extent to which calcidiol monohydrate is bioavailable as compared to native vitamin D_3, as well as to derive a conversion factor that allows to convert absolute amounts in μg of this nutrient form into μg of vitamin D_3.

[6] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. OJ L 304, 22.11.2011, p. 18.

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1.3. Overview of previous assessments of the UL for vitamin D

On 26 June 2012, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) expressed an opinion on the Tolerable Upper Intake Level (UL) for vitamin D (EFSA NDA Panel, 2012b). The NDA Panel derived a UL of 100 μg/day for adults, including pregnant and lactating women, that covered vitamin D intake from all food sources, including supplements. The same UL applies to children aged 11–17 years, while for children aged 1–10 years a UL of 50 μg/day was set, taking into account their smaller body size. For infants, the NDA Panel retained the UL of 25 μg/day previously set by the Scientific Committee on Food (SCF, 2003).

On 28 June 2018, the NDA Panel issued an update of the UL for vitamin D for infants, in which the UL of 25 μg/day set in 2012 for infants aged up to 6 months was retained, and a UL of 35 μg/day for infants 6–12 months was set (EFSA NDA Panel, 2018).

An overview of ULs for vitamin D established by risk assessment bodies is tabulated in Table 1 below. For a more detailed summary see Appendix A of the Protocol (Annex A).

ULs for vitamin D in adults have been established using persistent hypercalcaemia as the critical endpoint, as observed in human intervention studies. However, the key studies selected by IOM (Heaney et al., 2003), EFSA (Barger-Lux et al., 1998; Heaney et al., 2003) and the NHMRC (Vieth et al., 2001) for that purpose differ. Owing to the little data available, ULs for children and adolescents have been set as for adults NHMRC (2006), or scaled down from ULs for adults based on lower body weight and physiological considerations for children IOM (2011), or for children and young adolescents (EFSA NDA Panel, 2012b; SACN, 2016).

Table 1: Overview of existing UL values for vitamin D (μg/day)

Population group	EFSA NDA Panel (2012b, 2018)	IOM (2011)	NHMRC (2006)	SACN (2016)
Infants				
0–6 months	25	25	25	25
7–11 months[(1)]	35	38	25	25
Children and adolescents				
1–3 years	50	63	80	50
4–6 years	50			50
4–8 years		75	80	
7–10 years	50			50
9–13 years		100	80	
11–14 years	100			100
14–18 years		100[(2)]	80[(2)]	
15–17 years	100[(2)]			100
Adults				
≥ 18 years	100[(2)]			100
≥ 19 years		100[(2)]	80[(2)]	

(1): Age range covers the second half of the first year of life, i.e. from the beginning of the 7th month to the 1st birthday.
(2): Includes pregnant and lactating women.

1.4. Other assessments of vitamin D by EFSA

Dietary reference values

Following a request from the European Commission, EFSA derived dietary reference values (DRVs) for vitamin D for the European population (EFSA NDA Panel, 2016). The NDA Panel considered serum 25(OH)D concentration as an appropriate biomarker for vitamin D status in adult and children populations, and that it can also be used as a biomarker of vitamin D intake in a population with low exposure to UV-B irradiation. The Panel reviewed the available evidence on serum 25(OH)D concentration and musculoskeletal health outcomes, as well as pregnancy-related health outcomes. The Panel also considered several other non-musculoskeletal health outcomes (e.g., cancer and cardiovascular disease) that were reviewed in the IOM report, but without undertaking a specific literature search of primary studies.

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The Panel considered that at serum 25(OH)D concentrations below 50 nmol/L, there is evidence for an increased risk of adverse musculoskeletal health outcomes for adults, infants and children, and increased risk of adverse pregnancy-related health outcomes for pregnant women. The available studies which assessed the relationship between vitamin D intake and musculoskeletal health outcomes did not generally provide information on the habitual dietary intake of vitamin D, and the extent that cutaneous vitamin D synthesis has contributed to vitamin D supply, therefore the Panel concluded these studies were not useful for setting DRVs for vitamin D.

The Panel concluded that for setting DRVs for vitamin D, the dietary intake of vitamin D necessary to achieve a serum 25(OH)D concentration of 50 nmol/L is a suitable target value for all age and sex groups, assuming that intakes of interreacting nutrients (i.e., calcium) are adequate.

The assessment also included 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), which was carried out on data collected under conditions of assumed minimal cutaneous vitamin D synthesis.

The Panel considered that the available evidence did not allow the setting of average requirements (ARs) and population reference intakes (PRIs), and therefore defined adequate intakes (AIs) instead, for all population groups.

For adults, including pregnant and lactating women, and for children aged 1–17 years, the Panel set an AI for vitamin D at 15 μg/day. For adults, this was based on the meta-regression analysis, and considering that, at this intake, the majority (> 95%) of the adult population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L. For children, it was based on the meta-regression analysis of 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 set an AI for vitamin D at 10 μg/day, which was based on four trials that assessed the effect of vitamin D supplementation on serum 25(OH)D concentrations in (mostly) breastfed infants.

The Panel noted that the abovementioned AIs are under conditions of assumed minimal cutaneous vitamin D synthesis. In the presence of endogenous cutaneous vitamin D synthesis, the requirement for dietary vitamin D is lower or may be even zero.

Other assessments (OpenFoodTox, link)

25-Hydroxycholecalciferol monohydrate (25(OH)D_3), also called calcidiol, calcidiol monohydrate, calcifediol, or 25-hydroxy vitamin D_3, was assessed by the NDA Panel as novel food pursuant to Regulation (EU) 2015/2283[7] (EFSA NDA Panel, 2021a). The NDA Panel concluded that calcidiol monohydrate is safe under the proposed conditions of use (as food supplement) and use levels (up to 10 μg per day) for individuals ≥ 11 years old, including pregnant and lactating women.

Also in the context of novel food applications, the NDA Panel assessed the food ingredients' mushroom powder and baker's yeast that were exposed to ultraviolet irradiation to induce the conversion of provitamin D_2 to vitamin D_2. In both scenarios, the Panel concluded that the novel food was safe under the proposed conditions of use and use levels for the proposed target populations (EFSA NDA Panel, 2014, 2020, 2021b,c).

The safety of 25-hydroxycholecalciferol was also evaluated in the context of its use in animal feed by the EFSA FEEDAP Panel (EFSA FEEDAP Panel, 2005, 2009). The assessment included the safety for target species, consumers, users and the environment. The Panel suggested a provisional UL for 25(OH)D_3 (10 μg/day in adults, 5 μg/day in children), which was estimated using a biological activity factor relative to vitamin D_3 of 5 applied to the previous ULs for vitamin D available at the time (i.e., 50 μg/day in adults and 25 μg/day in children up to the age of 11; (IOM, 1997; SCF, 2003). The Panel concluded that under the proposed maximum doses, exposure resulting from the use of 25(OH)D_3 in animal feed would not present a risk for the consumer.

The FEEDAP Panel assessed the safety of vitamin D_3 in the context of its use in animal feed and considered that the use of vitamin D in animal nutrition, under the currently authorised maximum dietary content, has not and will not cause the UL to be exceeded (EFSA FEEDAP Panel, 2012, 2013, 2014, 2017).

[7] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001.

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1.5. Interpretation of the terms of reference and context of the assessment

According to the mandate, EFSA has first reviewed the guidelines of the SCF for the development of tolerable upper intake levels for vitamins and minerals (SCF, 2000). A draft guidance has been endorsed by the NDA Panel and published for a 1-year pilot phase (EFSA NDA Panel, 2022), after which it will be revised and complemented as necessary, following a public consultation.

The Panel interprets that the UL for vitamin D should be revised according to the principles laid down in the above-mentioned guidance, and that the mandate covers:

a) all forms of vitamin D authorised for addition to foods and for use in food supplements in the European Union (EU) (cholecalciferol and ergocalciferol), plus 25-hydroxycholecalciferol monohydrate (i.e., calcidiol monohydrate), from all dietary sources, i.e. foods (including fortified foods), beverages (including water), and food supplements.
b) an assessment of the relative bioavailability of calcidiol monohydrate as compared to cholecalciferol in order to provide a conversion factor for all population groups, if data allow doing so.

The Panel considers that the UL for vitamin D for infants recently revised (EFSA NDA Panel, 2018) does not need to be updated in the context of the current mandate.

In this opinion, the term vitamin D refers to cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2) and calcidiol monohydrate unless the specific form is indicated.

2. Data and methodologies

A protocol has been developed for this assessment (Annex A).

In accordance with the draft NDA Panel guidance on establishing and applying tolerable upper intake levels for vitamins and essential minerals (EFSA NDA Panel, 2022), the assessment questions underlying the UL evaluation are as follows:

- What is the maximum level of total chronic daily intake of vitamin D (from all sources) which is not expected to pose a risk of adverse health effects to humans? *(Hazard identification and characterisation)*
- What is the daily intake of vitamin D from all dietary sources in EU populations? *(Intake assessment)*
- What is the risk of adverse effects related to the intake of vitamin D in EU populations, including attendant uncertainties? *(Risk characterisation)*

Priority adverse health effects, i.e., those that are expected to play a critical role for establishing a UL, were identified in consultation with a panel of qualified experts on vitamin D[8] and after discussion by the ULs Working Group as follows: (a) persistent hypercalcaemia and hypercalciuria, and (b) musculoskeletal health, including risk of falling and risk of bone fractures in older adults, as well as bone mineral density (BMD), bone mineral content (BMC) and indices of bone strength at all ages. These have been addressed through systematic reviews of the literature. The rationale for the prioritisation of these adverse health effects is detailed in the protocol (Annex A). In addition, the relative bioavailability of calcidiol monohydrate ($25(OH)D_3$) compared to native vitamin D_3 has been addressed systematically to derive a conversion factor (CF) (EFSA ANS Panel, 2018).

The assessment of sub-questions identified as the result of the problem formulation, together with the methods selected to address them, are provided in Table 2.

[8] The expert panel was composed of Kevin Cashman (School of Food & Nutritional Sciences, University College Cork, Ireland) and Susan Lanham-New (Department of Nutritional Sciences, University of Surrey, UK).

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Table 2: Assessment sub-questions and methods to address them

No.	Sub-question	Methods
sQ1	**ADME of the different forms of vitamin D**	
	sQ1a. What is the ADME of the different forms of vitamin D in humans?	Narrative review
	sQ1b. Are there differences related to age, vitamin D status, or other individual factors, e.g. genetic polymorphisms?	Narrative review
	sQ1c. What is the relative bioavailability of calcidiol monohydrate (25(OH)D_3) compared to native vitamin D_3? Could a conversion factor be derived for use across population groups and vitamin D_3 doses?	Systematic review
sQ2	**Biomarkers of exposure for vitamin D**	
	sQ2a. What is the dose–response relationship between vitamin D intake and serum 25(OH)D concentrations?	Narrative review
	sQ2b. Are there differences related to age, sex, or other individual factors (e.g. genetic polymorphisms?)	Narrative review
sQ3	**Persistent hypercalcaemia/hypercalciuria**	
	sQ3a. Can a dose–response relationship between "high" vitamin D intake (and serum 25(OH)D) and risk of persistent hypercalcaemia/hypercalciuria in humans be characterised?	Systematic review
	sQ3b. What are the mechanisms by which "high" vitamin D intake (and serum 25 (OH)D) could increase the risk of persistent hypercalcaemia/hypercalciuria in humans?	Narrative review
sQ4	**Musculoskeletal health**	
	sQ4a. What is the relationship between "high" vitamin D intake (and serum 25 (OH)D) and risk of bone fractures (all sites) in older adults? Could a dose–response be characterised?	Systematic review
	sQ4b. What is the relationship between "high" vitamin D intake (and serum 25 (OH)D) and risk of falling (i.e. risk of falls, risk of falling at least once, or both) in older adults? Could a dose–response be characterised?	Systematic review
	sQ4c. What is the relationship between "high" vitamin D intake (and serum 25 (OH)D) and BMD/BMC and indices of bone strength (all sites) in humans? Could a dose–response be characterised?	Systematic review
	sQ4d. What are the mechanisms by which "high" vitamin D intake (and serum 25 (OH)D) could increase the risk of falling/bone fractures and/or decrease BMD in humans?	Narrative review
sQ5	What **other adverse health effects** have been reported to be associated with 'high' intake of vitamin D?	Narrative review
sQ6	**Vitamin D intake**	
	sQ6a. What are the levels of vitamin D in foods, beverages and food supplements in the EU?	Food composition and food consumption data in the EU
	sQ6b. What is the distribution of intakes of vitamin D from all dietary sources (including fortified foods and food supplements) by population group in the EU?	

sQ: sub-question.

The preparatory work for this assessment was contracted out by EFSA through a call for tender (OC/EFSA/NUTRI/2021/01) (see Section 1 of the Protocol for more details). The preparatory work to address sub-question (sQ) 1 to sQ5 has been carried out by the University of Helsinki, in collaboration with the University of Oslo, and the technical report has been published (Lamberg-Allardt et al., 2023). The Panel made an independent evaluation of the evidence and adapted the outcome of the contractor's work where needed.

A draft opinion was endorsed by the NDA Panel on 29 March 2023 and was open for public consultation from 24 April to 5 June 2023. The draft opinion has been amended in view of the comments received, which have all been addressed and are published in a technical report (Annex E).

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2.1. Hazard identification and characterisation

2.1.1. Data

For sub-questions addressed through narrative reviews, textbooks, authoritative reviews and research papers have been retrieved through non-systematic searches in bibliographic databases.

For sub-questions addressed through systematic reviews a brief description of the processes used for evidence retrieval, study selection, and data extraction is provided below. More information on these steps is available in the contractor's technical report (Lamberg-Allardt et al., 2023).

2.1.1.1. Literature searches

To address sQ1c, sQ3a and sQ4a/b/c, relevant human studies on the selected endpoints were identified by the University of Helsinki as contractor through systematic searches of the literature in MEDLINE (Ovid), Embase (Ovid) and Cochrane Central Register of Controlled Trials. Searches were targeting articles published in English only. The search strategy was developed by information specialists from the Karolinska Institutet, in collaboration with the contractor, and peer reviewed by information specialists at the University of Oslo and EFSA. Specific search strings were used in the aforementioned databases to limit by type of study and publication type, and with no date limitations, as described in the protocol (Annex A of the opinion). The search strategy is further detailed in Annex B of the technical report (Lamberg-Allardt et al., 2023). The searches covered literature published up to March 17, 2022 for sQ1, March 14, 2022 for sQ3a, and March 25, 2022 for sQ4a/b/c.

The literature searches for sQ4 (musculoskeletal health) were designed to address each type of endpoint (i.e., fractures, falls, BMD/BMC and indices of bone strength). The results by endpoint and database were combined.

2.1.1.2. Study selection

Articles retrieved were screened in duplicate in Distiller SR® (Web-Based Systematic Review Software; Evidence Partners, Ottawa, Canada) according to the eligibility criteria defined in the protocol (Annex A). Conflicts were resolved by discussion or by a third reviewer. For the title and abstract screening step, the artificial intelligence tool built in Distiller SR® was also used. To maximise the identification of relevant publications, the reference list of systematic or narrative reviews identified via the search were scrutinised for additional eligible studies. Reviews, expert opinions, editorials, letters to the editors, abstracts, posters, theses and grey literature (i.e. literature not indexed in literature databases) were excluded.

The eligibility criteria for the selection of human studies on sQ1c, sQ3a and sQ4a/b/c are listed in Sections 3.2.2, 3.3.1.1 and 3.3.1.2 of the protocol, respectively (Annex A). After discussion with the contractor, the decision was taken to deviate from the protocol and exclude studies in which the highest dose of supplemental vitamin D investigated was < 15 μg/day for sQ3a and sQ4a/b/c because such intakes are lower than the current adequate intake for vitamin D in adults and thus would not meaningfully contribute to the body of evidence in the context of a UL assessment (EFSA NDA Panel, 2012b).

For all sQs, randomised controlled trials (RCTs) on vitamin D using a supplementation pattern less frequent than weekly were excluded, owing to the uncertainties associated to the extrapolation of the results from these studies to the health effects of daily doses of vitamin D, which are the basis for deriving DRVs, including ULs.

Briefly, for sQ1c on the conversion factor for calcidiol monohydrate, the inclusion criteria were restricted to human RCTs and non-randomised comparative studies of interventions with a study duration of at least 6 weeks and with no population restriction. Only studies comparing oral supplementation with vitamin D_3 versus calcidiol monohydrate (with or without a co-intervention that was the same for both arms) were included. The endpoint of interest was plasma or serum 25(OH)D concentrations (referred to as serum concentrations hereafter for simplicity) and all methods of measurement were included. Parathyroid hormone (PTH) was also of interest in studies that reported on serum 25(OH)D. A total of 4,304 records were identified after removing duplicates and screened at title and abstract level, of which 43 full-text papers were assessed for eligibility and 16 were included. During data extraction, 4 additional references were excluded because of duplication, leaving a total of 12 publications reporting original data (Appendix A, Figure A.1).

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As for sQ1c, the same inclusion criteria in relation to study design and duration was applied for sQ3a on hypercalcaemia/hypercalciuria. Eligible study populations were those aged > 1 year because the UL for infants was recently re-evaluated by EFSA (EFSA NDA Panel, 2018) and it is out of the scope of this assessment. Exclusion criteria were clinical conditions leading to persistent hypercalcaemia/hypercalciuria unrelated to vitamin D intake (e.g. primary hyperparathyroidism, cancer, idiopathic hypercalciuria). Studies were eligible for inclusion if they investigated oral vitamin D supplementation at doses ≥ 15 μg/day versus placebo or lower vitamin D doses. Studies that investigated vitamin D with a co-intervention were only eligible if controlling for the co-intervention (e.g., calcium added to all vitamin D arms and placebo). The endpoints of interest were persistent hypercalcaemia and hypercalciuria, as defined by the authors. Studies that measured calcium in blood and/or urine only once after baseline were not eligible. After removing duplicates, a total of 8,277 records were identified for sQ3a (hypercalcaemia/hypercalciuria) and screened at the title and abstract level. Of these, 262 full-text papers were assessed for eligibility, and in addition one paper was identified via citation searching, resulting in 82 papers that met the eligibility criteria. After exclusion at data extraction level, 37 publications reporting on 34 studies were included in the assessment (Appendix A, Figure A.2). The publications by Gallagher et al. (2012, 2014a)reported results on different endpoints from the same RCT. Henceforth, Gallagher et al. (2012) will be referenced in this opinion although information may have been extracted from the later publication. In addition, Gallagher et al. (2012, 2013) both report on the same trial, but results have been analysed and published separately by race. Jorde et al. (2008) and Sneve et al. (2008) refer to the same RCT, and subsequently the former publication will be referenced. The list of RCTs reported in multiple publications can be found in Appendix D.

For sQ4a (fractures) and sQ4b (falls), only human RCTs and non-randomised comparative studies of interventions with a study duration of at least 12 months were eligible. The population of interest was older men (aged 55 years and over) and post-menopausal women (as defined by the authors). The age cut-off for males was defined to be as inclusive as possible for RCTs investigating these endpoints. Studies on individuals with primary hyperparathyroidism or other disorders affecting musculoskeletal health were not eligible. The same eligibility criteria as for sQ3a were applied in relation to exposure. Endpoints of interest were bone fractures (all sites), either self-reported or diagnosed by a physician, falls as defined by the authors, and composite indices thereof.

For sQ4c (BMD/BMC and bone strength), in addition to the study designs described above for sQ4a/b, prospective studies (cohort, case-cohort, and nested case–control) were also eligible. The same eligibility criteria for study duration and exposure were applied. In addition, prospective studies investigating serum 25(OH)D as a biomarker of exposure were also eligible. Only studies that measured BMD/BMC by dual-energy x-ray absorptiometry (DXA) or peripheral quantitative computed tomography (pQCT), and measures of bone strength derived from pQCT, were eligible.

For sQ4 (musculoskeletal health), a total of 15,541 unique records were identified after removing duplicates and screened at title and abstract level. At full-text screening, 210 papers were assessed for eligibility of which 54 met the inclusion/exclusion criteria. Of these, 20 publications reporting on 16 studies were included in the assessment, of which 8 reported on fractures, 11 on falls and 7 on BMD, BMC and/or bone strength indices (Appendix A, Figure A.3).

Reasons for references excluded at full-text screening, or during data extraction, are outlined in Annexes D, H and M of the technical report (Lamberg-Allardt et al., 2023).

2.1.1.3. Data extraction

Data were extracted into Microsoft Excel® by two extractors and were jointly discussed, compared and harmonised at several time points. Evidence tables were prepared in Microsoft Word® and are provided in Appendix C.

For sQ3a, data extraction was restricted to studies with at least one arm with a vitamin D dose ≥ 100 μg/day in adults and ≥ 50 μg/day in children (i.e., at or above the current UL for vitamin D for the respective population groups) because in the publications identified with vitamin D supplements below these values, cases of hypercalcaemia or hypercalciuria did either not occur, were not persistent, and/or could not be related to the vitamin D dose administered (i.e., the treatment group was not specified in the publication, the number of cases was higher at lower doses of vitamin D, and/or persistent cases occurred in patients with primary hyperparathyroidism). Hence the publications were considered not useful in establishing a UL. Of the 80 papers meeting the eligibility criteria, data were not extracted for 45 of these papers because intervention doses were < 100 μg/day for adults

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or < 50 μg/day for children (n = 31) or otherwise data were duplicates (n = 14). These studies were included in the assessment but were only narratively summarised.

For sQ4c, the following decisions for data extraction were made after mapping the results of the eligible studies:

a) data from prospective cohort studies on the relationship between serum 25(OH)D concentration and BMD/BMC/indices of bone strength (n = 16) were not extracted because no adverse effects were reported at higher serum 25(OH)D concentrations on the endpoints, rather the opposite (see Section 3.5.2.3).
b) as for sQ3a, data extraction from RCTs was limited to studies with at least one dose at or above the current UL for vitamin D because no adverse effects of vitamin D supplementation were reported on the endpoints at lower doses (n = 14) (see Section 3.5.2.3).

For further details on data collection and preparation methods see the technical report (Lamberg-Allardt et al., 2023).

2.1.1.4. Requests for additional information

Additional data were requested from study authors when this information was pertinent to the interpretation of the study results. See Annex C of the technical report (Lamberg-Allardt et al., 2023) for details on data requested.

2.1.2. Methodologies

The methodology for this assessment follows the guidance for establishing ULs developed by the NDA Panel (EFSA NDA Panel, 2022). EFSA's transversal guidance for use in scientific assessments in relation to the application of the systematic review methodology in food and feed safety (EFSA, 2010), the principles and processes for dealing with data and evidence (EFSA, 2015b), the assessment of the biological relevance of data (EFSA Scientific Committee, 2017a), the use of weight of evidence (EFSA Scientific Committee, 2017b), the appraisal and integration of evidence from epidemiological studies (EFSA Scientific Committee, 2020), and the analysis of uncertainty in scientific assessments (EFSA Scientific Committee, 2018), have also been considered.

The methodology used for the appraisal of the internal validity of included studies from the systematic reviews, for evidence synthesis and integration, and for the analysis of uncertainty in the context of this assessment, is described below.

2.1.2.1. Evidence appraisal (sQ1c, sQ3, sQ4)

The internal validity of eligible studies for which data were extracted in relation to sub-questions 1c, 3a and 4a/b/c (i.e., addressed through systematic reviews) was assessed in duplicate by two independent reviewers using a customised version of the Office of Health Assessment and Translation (OHAT) risk of bias (RoB) tool developed by the US National Toxicology Program (NTP) (OHAT-NTP, 2015). Any discrepancies in the RoB assessment for each bias domain were discussed among the assessors. If there was disagreement, a third reviewer was consulted for resolution.

The appraisal addressed eight RoB questions for intervention studies, covering seven domains. The questions considered the most critical for the allocation of studies to RoB tiers (key questions) were those related to randomization and those related to detection bias in the exposure and outcome. In accordance with the OHAT/NTP guidelines, the RoB tool was customised to fit the specific nature of the review questions. The default OHAT/NTP tiering approach, which combines the evaluations of all the RoB questions into an overall RoB judgement (i.e., low (tier 1), moderate (tier 2) or high (tier 3) RoB), was also modified (Table 3). The OHAT RoB tool proposes five response options for each RoB question: definitely low RoB (++), probably low RoB (+), not reported (NR), probably high RoB (−), definitely high RoB (−−).

Table 3: Modified version of the OHAT pre-defined algorithm

Tier 1	Study must be rated as "definitely low" ++ or "probably low" + risk of bias for all *key criteria* AND have most other applicable criteria rated as "definitely low" ++ or "probably low" + risk of bias.	**Low RoB**
Tier 2	Study does not meet criteria for Tier 1 or Tier 3.	**Moderate RoB**

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Tier 3	Study must be rated as "definitely high" -- or "probably high" -/NR risk of bias for most (at least two) *key criteria* AND have most other applicable criteria rated as "definitely high" -- or "probably high" -/NR risk of bias.	**High RoB**

The forms used for the RoB assessment, including the explanations for expert judgements, can be found in Appendix C of the technical report (Lamberg-Allardt et al., 2023).

2.1.2.2. Evidence synthesis (sQ1c, sQ3, sQ4, sQ5)

For sQ1c on the conversion factor for calcidiol monohydrate, several meta-analyses were used for evidence synthesis. The heterogeneity of the effect size across studies was tested by the Q statistic and quantified by estimating the I^2 statistic. Sub-group analyses were carried out to explore potential sources of heterogeneity (methodological and contextual). Sensitivity analyses were conducted to examine the influence of specific assumptions on the overall effect size. Publication biases were assessed (e.g. by visual inspection of the funnel and by performing the Egger's test for funnel plot asymmetry). A dose–response meta-regression analysis was also conducted.

For sQ3 and sQ4, a narrative qualitative synthesis of the evidence was performed through descriptive forest plots when 3 or more studies were available for a given endpoint. A quantitative synthesis of the evidence through meta-analyses or dose–response analyses was not performed for these sQs, owing to the heterogeneity of the available data.

A narrative synthesis of the available evidence was performed for sQ5.

2.1.2.3. Evidence integration and uncertainty analysis (sQ1c, sQ3, sQ4, sQ5)

Conversion factor for calcidiol monohydrate (sQ1c)

The integration of the available evidence to derive a CF for calcidiol monohydrate and the analysis of the uncertainties associated to the proposed value are narratively discussed.

Hazard identification

The purpose of the hazard identification step is to assess the available evidence for a causal positive relationship between vitamin D intake and the risk of adverse health effects assessed.

Regarding sQ3a, a causal relationship between 'high' vitamin D intake and increased risk of hypercalcaemia and/or hypercalciuria is well-established, thus the assessment focused on the characterisation of the dose–response relationship. Uncertainties in the body of evidence are narratively described. No comprehensive uncertainty analysis is performed.

Regarding sQ4 and sQ5, the available body of evidence (BoE) did not suggest a positive relationship between high vitamin D intake or status and the adverse health effects assessed (i.e. the relationship appeared to be negative or null), and thus no formal evidence integration or uncertainty analysis were carried out for these sQs. Uncertainties in the BoE are, however, narratively discussed, particularly for sQ4.

Hazard characterisation

At this step, evidence is integrated to select the critical effect(s) and identify a reference point (RP) for establishing the UL. If the available data are not suitable for dose–response modelling, a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL) could be identified and used as the RP. ULs are derived for different life-stage groups using relevant data for each group, where available. The UL is derived as follows: UL = RP/UF, where UF is an uncertainty factor which accounts for the uncertainties associated with extrapolating from the observed data to the general population, as ULs should be protective for all members of the general population, including sensitive individuals, throughout their lifetime (EFSA NDA Panel, 2022). The rationale for the selection of the RP and UF is documented in the scientific opinion.

2.2. Dietary intake assessment

2.2.1. Data

Food intake data from the EFSA Comprehensive European Food Consumption Database (hereinafter referred as Comprehensive Database) and data on vitamin D content in foods from the EFSA food composition database (FCDB) were used.

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Food consumption data

The Comprehensive Database provides a compilation of existing national information on food consumption at individual level collected through repeated non-consecutive 24-h dietary recalls or dietary records (EFSA, 2011a,b). The latest version of the Comprehensive Database, updated in 2022, contains results from a total of 83 different dietary surveys carried out in 29 different European countries (including EU member states, pre-accession countries and the United Kingdom) covering 154,388 individuals. In this assessment, only food consumption surveys from 22 EU member states with information for at least 2 days per subject were used.

Food composition data

Composition data for total vitamin D in foods and beverages were derived from the EFSA Nutrient Composition Database, which was compiled as a deliverable of the procurement project "Updated food composition database for nutrient intake" (Roe et al., 2013). The EFSA food composition database contains data for energy, macro- and micronutrients from national food composition databases provided by 14 national food database compiler organisations covering ~ 1,750 food entries and harmonised information on the most common composite recipes of European countries up to 2012. When needed, publicly available national food composition databases and the Mintel Global New Products Database (GNPD)[9] were used to complement EFSA's FCDB.

The GNPD was further used as a data source to identify the type of vitamin D containing food supplements and fortified foods available on the EU market. The search was limited to the past five years, from November 2017 to November 2022.

More details on these data sources are described in Annex C of this opinion.

Other data sources

To complement EFSA's intake assessment, vitamin D intake estimates from natural sources, from addition to foods and from food supplements based on nationally representative food consumption surveys without date limits were collected between September and November 2021 by contacting 64 competent authorities in 37 European countries through EFSA Focal Points and the EFSA Food Consumption Network. An additional search in sources of bibliographic information (Google Scholar, PubMed) was performed to collect reports of national surveys included in the Comprehensive database that had not been obtained through the competent authorities. Between August and October 2022, EFSA contacted all EU Member States and Norway through the European Commission Working Group on Food supplements and Fortified foods and collected data on the intake of vitamin D specifically from food supplements. These data have been used to evaluate the accuracy of the results obtained, comparing EFSA's estimates with published national intake estimates from the same surveys with the same (or similar) window of data collection and population groups, when available (EFSA, 2022).

2.2.2. Methodologies

Intake assessment from natural sources

The FoodEx2 classification and description system was used to facilitate the linkage between the food consumption and food composition databases (EFSA, 2015a). Food consumption and composition data used in the assessment were checked for consistency of FoodEx2 codes and the original food name in English (freely entered text).

The plausibility of amounts consumed and of total vitamin D content of each given foodstuff was checked when outlying values were observed. As the scope of intake assessment was to consider only natural sources of vitamin D, consumption and composition data on food supplements were disregarded, and the composition database was extensively cleaned to exclude fortified foods. Since vitamin D is sensitive to heat treatment of foods, retention factors were applied to adjust the vitamin D content in foods for losses during cooking (Vásquez-Caicedo et al., 2008).

Dietary intakes of vitamin D in μg/day from natural food sources were calculated linking food consumption data at individual level to food composition data. The resulting intakes per food item were summed up to obtain total daily intakes of vitamin D for each individual. The mean, P5, median

[9] The Mintel GNPD contains information on over three million food and beverage products, of which more than one million are or have been available on the European food market. Twenty five out of the 27 EU Member States and Norway are present in the database. The database provides the compulsory ingredient information reported on product labels and the nutrition declaration when available. https://www.mintel.com/globalnew-products-database

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and P95 of intakes were subsequently calculated for each survey by population group and sex, as well as total populations.

The methodology followed for the assessment of intake from natural sources is further detailed in Annex C.

Intake assessment from fortified foods and food supplements

Data on the intake of vitamin D from recent national food consumption surveys, including specific estimates of intake from food supplements and/or fortified foods, were extracted and are provided in Annex D.

Information on food products fortified with vitamin D and vitamin D-containing supplements available on the EU market, and vitamin D content as reported on the label, were extracted from the Mintel GNPD. These data were used qualitatively to describe the types of fortified foods and food supplements available and to gain insight into their potential contribution to total vitamin D intake.

3. Assessment

The assessment refers to all forms of vitamin D currently authorised for addition to foods and food supplements in the EU (i.e. cholecalciferol and ergocalciferol) plus calcidiol monohydrate. In this opinion, the term vitamin D refers to cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2) and calcidiol monohydrate unless the specific form is indicated. Vitamin D intake is expressed in μg and concentrations in blood are expressed in nmol/L.[10]

In the body, vitamins D_2 and D_3 are converted to the main circulating forms, 25(OH)D_2 and 25(OH)D_3, called calcidiols, and these to the active metabolites 1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$, called calcitriols. In this opinion, the term 25(OH)D refers to both 25(OH)D_2 and 25(OH)D_3, and 1,25$(OH)_2$D refers to both 1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$ unless the specific form is indicated.

3.1. Chemistry of vitamin D

Vitamin D 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) upon exposure to ultraviolet-B (UV-B) irradiation that opens the B-ring of the molecules, and subsequent thermal isomerisation. Vitamin D_2 ($C_{28}H_{44}O$) differs from vitamin D_3 ($C_{27}H_{44}O$) in the side chain where it has a double bond between C22 and C23 and an additional methyl group on C24 (EFSA NDA Panel, 2016). Calcidiol monohydrate ($C_{27}H_{46}O_3$) is obtained by chemical synthesis (EFSA NDA Panel, 2021a) and differs from 25-hydroxyvitamin D_3 ($C_{27}H_{44}O_2$), the main metabolite of vitamin D_3 in the circulation, by a water molecule (Figure 1). Calcidiol monohydrate and 25-hydroxyvitamin D_3 are known to be functionally equal and are often used as synonymous.

[10] For conversion between nmol/L and ng/mL for serum 25(OH)D concentration: 2.5 nmol/L = 1 ng/mL. For conversion between μg and International Units (IU) of vitamin D intake (vitamins D_2 and D_3): 1 μg = 40 IU and 0.025 μg = 1 IU.

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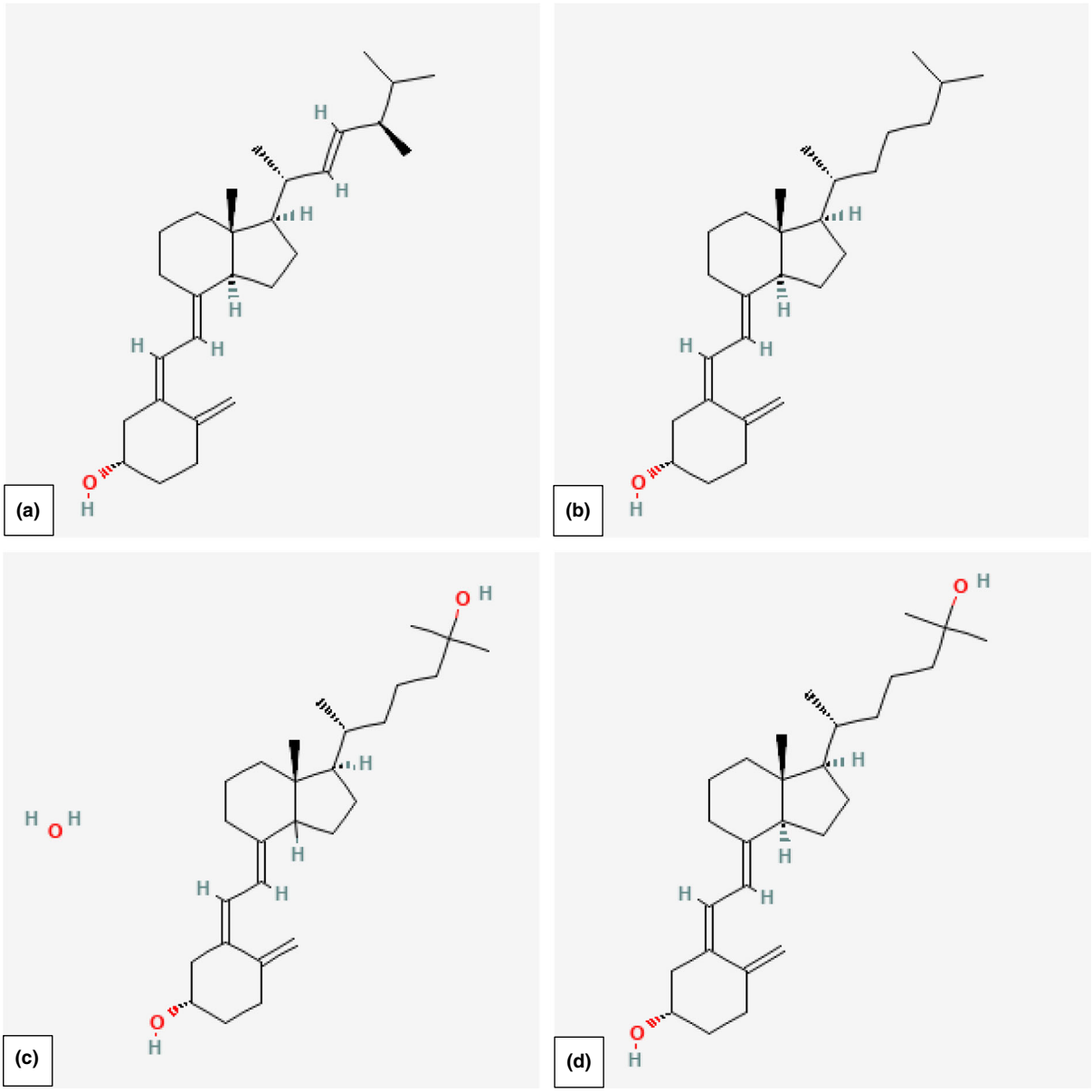

Figure 1: Chemistry of vitamin D

Ergocalciferol (a), cholecalciferol (b), calcidiol monohydrate (c), and 25-hydroxyvitamin D_3 (d). Source: PubChem (CID 5280793, 5280795, 6441383 and 5283731)[11]

Authorised forms of vitamin D for addition to foods[2] and for use in food supplements[1] for human use in the EU are reported in Table 4.

Table 4: Forms of vitamin D authorised as nutrient sources for human use in the EU

	Addition to foods *Regulation (EC) 1925/2006*[2]	**Food supplements *Directive 2002/46/EC*[1]**
Cholecalciferol	x	x
Ergocalciferol	x	x

[11] https://pubchem.ncbi.nlm.nih.gov/compound/5280793#section=2D-Structure
https://pubchem.ncbi.nlm.nih.gov/compound/5280795#section=2D-Structure
https://pubchem.ncbi.nlm.nih.gov/compound/6441383#section=2D-Structure
https://pubchem.ncbi.nlm.nih.gov/compound/5283731#section=2D-Structure.

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3.2. Physiology and metabolism

3.2.1. Cutaneous synthesis

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. Immediately after its formation, previtamin D_3 thermally isomerises to vitamin D_3 in the lower layers of the skin (Engelsen et al., 2005). Environmental (e.g. latitude, season) and individual (e.g. type of skin, time spent outdoors, use of sunscreen, clothing, age) factors affecting skin synthesis of vitamin D_3 have been extensively reviewed by EFSA (EFSA NDA Panel, 2016).

Downregulation of vitamin D synthesis in the skin through UV-B radiation exposure is the mechanism by which vitamin D toxicity due to prolonged sun exposure is prevented (EFSA NDA Panel, 2016). When (pre)vitamin D_3 is exposed to solar UV-B radiation, it is converted to a variety of photoproducts that have insignificant activity on calcium metabolism, such as tachysterol, lumisterol or suprasterol (Holick, 1988; Bouillon et al., 1998). However, there is no information that dietary vitamin D_2, vitamin D_3 or calcidiol would influence dermal synthesis of vitamin D_3, and sun exposure contributes a considerable and varying amount of vitamin D available to the body (Wacker and Holick, 2013). Therefore, the Panel notes that sun exposure should be considered when addressing the relationship between vitamin D intake and adverse health effects for the purpose of setting a UL.

3.2.2. Intestinal absorption

Vitamin D_2 and vitamin D_3 are fat-soluble and are present in foods, including fortified foods, and food supplements (see Section 3.4.1). Calcidiol is naturally present in some foods of animal origin in varying amounts (Cashman, 2012). Calcidiol produced by chemical synthesis is used in food supplements (Bischoff-Ferrari et al., 2012; Vaes et al., 2018b; EFSA NDA Panel, 2021a).

After oral intake, vitamins D_2 and D_3 from foods and food supplements are absorbed throughout the small intestine with an efficiency varying between 55 and 99% (mean about 80%), with no discrimination between vitamins D_2 and D_3 (Thompson et al., 1966; Lo et al., 1985; Jones, 2014; Borel et al., 2015; Reboul, 2015; EFSA NDA Panel, 2018). Absorption of vitamins D_2 and D_3 occurs mostly in the distal small intestine and is dependent on the presence of bile acids and micelle formation. Vitamins D_2 and D_3 are then incorporated into chylomicrons, which reach the systemic circulation through the lymphatic system (Quesada-Gomez and Bouillon, 2018). Limited data are available on the effect of the food or supplement matrix on absorption of vitamins D_2 or D_3. Whereas it has been suggested that the absorption process is more efficient in the presence of dietary fat in the lumen of the small intestine and when ingested with a meal (Dawson-Hughes et al., 2015), the food matrix appears to have little effect on vitamins D_2 and D_3 absorption efficiency (Borel et al., 2015). In a recent randomised cross-over trial, no difference was observed between milk and water as vehicles for vitamin D_3 supplements (Espersen et al., 2023). Data also suggests that age *per se* has no effect on vitamin D absorption efficiency (Borel et al., 2015).

Intestinal absorption of the hydroxylated form of vitamin D_3 calcidiol does not require the presence of bile acids and micelle formation, and thus is faster and more efficient (about 93%, even in individuals with fat malabsorption) than that of the non-hydroxylated vitamins D_2 and D_3 (Borel et al., 2015; Janousek et al., 2022). After intestinal absorption, calcidiol reaches the systemic circulation via the portal vein (Quesada-Gomez and Bouillon, 2018).

3.2.3. Transport in blood

Vitamin D_3 from dermal synthesis is transported in plasma bound to the specific vitamin D–binding protein (DBP), whereas dietary vitamins D_2 and D_3 (from food and supplements) are transported in chylomicrons, with some transfer to DBP. 25(OH)D resulting from hydroxylation of vitamins D_2 and D_3 primarily in the liver (see Section 3.2.6) and from the intestinal absorption of calcidiol is transported in blood bound to DBP (85–90%), albumin (10–15%) or free ($< 1\%$). Hydroxylation of 25(OH)D mainly in the kidney (see Section 3.2.6) leads to $1,25(OH)_2D$, which is primarily transported bound to DBP and to a lesser extent to albumin (EFSA NDA Panel, 2016).

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3.2.4. Distribution to tissues

Vitamin D from the diet is released from chylomicrons at arrival tissues by action of the enzyme lipoprotein lipase, either for hydroxylation (e.g. liver) or storage. Serum 25(OH)D and $1,25(OH)_2D$ are released from DBP to various tissues, including bone, intestine, kidney, pancreas, brain and the skin. 25(OH)D is taken up from the blood into tissues probably by protein-binding, whereas $1,25(OH)_2D$, the active metabolite of vitamin D, binds to the intracellular vitamin D receptor (VDR).

3.2.5. Storage

Adipose tissue is the main storage site of vitamin D in the body. Vitamin D is also long-term stored in muscle, liver, and other tissues (Blum et al., 2008; Heaney et al., 2009). 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). About 75% of vitamin D_3 is stored in adipose tissue, whereas 25(OH)D is more evenly distributed through the body (approximately 35% in adipose tissue, 30% in blood, 20% in muscle and 15% in other tissues) (Heaney et al., 2009).

3.2.6. Metabolism

Bioactivation of vitamins D_2 and D_3 requires two steps, whereas only the second step is needed for calcidiol monohydrate.

The first step entails 25-hydroxylation to 25(OH)D after vitamin D is released from DBP primarily in the liver, although several other tissues express this enzymatic activity. 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, 2014). In mouse knockout studies and in humans with mutations in these enzymes, only CYP2R1 loss is associated with decreased 25(OH)D concentrations. The mitochondrial 25-hydroxylase CYP27A1 was first identified as catalysing a critical step in the bile acid synthesis pathway (Bikle, 2021). 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 mostly bound to DBP (Section 3.2.3) and transported to the kidneys (Quesada-Gomez and Bouillon, 2018).

The second step is the 1α-hydroxylation of 25(OH)D to $1,25(OH)_2D$ by a P450 enzyme, the 25-hydroxyvitamin D 1-α-hydroxylase CYP27B1, which mostly occurs in the kidneys. 25(OH)D can enter the renal tubuli as free form via the bloodstream or bound to DBP, the uptake of which is mediated by megalin/cubulin after filtration in the glomeruli. The kidney is the only tissue producing $1\alpha,25(OH)_2D$ with a systemic action, whereas in other organs, such as bone, the parathyroid glands, and the placenta, the synthesis of $1,25(OH)_2D$ is only for autocrine/paracrine cell activities and depends on the availability of free 25(OH)D in blood and the extra-renal expression of CYP27B1. CYP27B1 activity in the kidney is mainly regulated by calcium, phosphate, PTH, fibroblast-growth factor 23 (FGF23), and $1,25(OH)_2D$ (Jones, 2014; EFSA NDA Panel, 2016).

Only $1,25(OH)_2D$ produced in the kidney reaches the bloodstream, in which it is transported bound to DBP and then released as free $1,25(OH)_2D$, which can access target cells, activate the nuclear receptor VDR, and thereby regulate gene transcription. A very large number of genes (~ 3% of the human genome) are under the direct or indirect control of the active hormone, suggesting a broad spectrum of activities. $1,25(OH)_2D$ may also activate nongenomic pathways (Bouillon et al., 2019). Although vitamins D_2 and D_3 are not discriminated by the specific vitamin D signal transduction cascade and are considered biologically equivalent in their ability to cure rickets (Jones, 2013), recent data from human transcriptome analyses has shown that gene expression related to immunity differs between vitamin D_2 and D_3 (Durrant et al., 2022).

25(OH)D and $1,25(OH)_2D$ are inactivated, primarily in the kidney, through 24-hydroxylation (C24 hydroxylation pathway), resulting in $24,25(OH)_2D$ and 1,24,25-trihydroxyvitamin D ($1,24,25(OH)_3D$), respectively. $24,25(OH)_2D$ prevents conversion of 25(OH)D to $1,25(OH)_2D$, whereas $1,24,25(OH)_3D$ leads to calcitroic acid (EFSA NDA Panel, 2016). The enzyme 25(OH)D-24 hydroxylase (CYP24A1) is reciprocally regulated (stimulated by $1,25(OH)_2D_3$ and suppressed by PTH), which tends to sustain blood $1,25(OH)_2D_3$ concentrations (Pike and Christakos, 2017). Human CYP24A1 also catalyses, although to a lesser extent, the 23-hydroxylation (C23 lactone pathway) both 25(OH)D and $1,25(OH)_2D$ leading, in sequential steps, to 25(OH)D-26,23-lactone and $1,25(OH)_2D$-26,23-lactone, respectively (Jones, 2014; Jones et al., 2014).

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Following vitamin D supplementation, CYP27A1 is upregulated with a lag of several weeks (Wagner et al., 2011). It has been suggested that, even if 24-hydroxylation remains the main path through catabolism and inactivation of vitamin D metabolites, 24,25$(OH)_2$D could have biologic effects different from 1,25$(OH)_2$D in some tissues, such as bone (Jones, 2014; Bikle, 2021).

3.2.7. Elimination

The majority (around 70%) of the metabolites of the vitamin D pathways of degradation (e.g. calcitroic acid) are excreted in the bile (Jones, 2014), and thus in faeces. Due to active renal reuptake, the urinary excretion of vitamin D metabolites is low (Jones, 2013; EFSA NDA Panel, 2016; Janousek et al., 2022). Breast milk only accounts for a small part of the vitamin D elimination in lactating women (EFSA NDA Panel, 2016).

3.3. Biomarkers of intake and status

3.3.1. Serum concentration of 25(OH)D as marker of vitamin D intake and status

Plasma or serum 25(OH)D concentrations (referred to as serum concentrations hereafter for simplicity) reflect the amount of vitamin D from both cutaneous synthesis and dietary sources, including supplements. Serum 25$(OH)D_2$ is of dietary origin only, while serum 25$(OH)D_3$ may be of dietary or dermal origin. Serum 25(OH)D has a long mean half-life of $\sim$ 13–15 days (Jones et al., 2014; Cesareo et al., 2019), is considered a useful marker of vitamin D status (Seamans and Cashman, 2009), and a biomarker of vitamin D intake in people with low exposure to UV-B irradiation from sunlight (EFSA NDA Panel, 2016).

3.3.1.1. Methods of measurement of 25(OH)D in plasma/serum

High variability in serum 25(OH)D measurements obtained with different analytical methods may hamper comparisons across studies, and characterisation of the dose–response between vitamin D intake and serum 25(OH)D concentrations. A summary of the methods available for the measurement of 25(OH)D in serum and of the efforts made to standardise serum 25(OH)D measurements can be found in previous EFSA opinions (EFSA NDA Panel, 2016, 2018).

Briefly, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is the golden standard. Alternatively, high-performance liquid chromatography (HPLC) is used in some laboratories. Both methods can measure serum 25$(OH)D_3$ and 25$(OH)D_2$ separately. LC–MS/MS can also measure other vitamin D metabolites, such as 24,25$(OH)_2$D or 3-epi-25-hydroxyvitamin D_3 (Wallace et al., 2010; Carter et al., 2018). Immunoassays (competitive protein binding assays (CPBA), enzyme-linked immunosorbent assays (ELISA/EIA), chemiluminescent immunoassays (CLIA)) are commercially available for routine use in epidemiological studies and the clinic.

The introduction of a standard reference material for vitamin D in human serum and the development of protocols for standardising procedures of 25(OH)D measurement by the Vitamin D Standardisation Program (VDSP)[12] have pushed forward comparability of the results obtained through different immunoassays commercially available for the measurement of total serum 25(OH)D concentrations (i.e. the sum of 25$(OH)D_2$ and 25$(OH)D_3$). In a recent interlaboratory study conducted in the framework of the VDSP, 12 different immunoassays (11 unique) were tested against LC–MS/MS using reference measurement procedures. Whereas immunoassays performed reasonably well in most serum samples (all met the performance criteria of $\leq$ 10% CV and 9 out of 12 were $\leq \pm$5% mean bias), 10 out of 12 showed changes in response in the eight samples with high concentrations of 25 $(OH)D_2$ ($>$ 30 nmol/L), suggesting that response or recovery for 25$(OH)D_2$ and 25$(OH)D_3$ may be unequal in some assays (Wise et al., 2021).

In this opinion, the method of measurement of serum 25(OH)D concentrations has not been used to exclude studies for relevance, but rather considered in the appraisal of the RoB for the outcome assessment (see Appendix C of the technical report).

[12] The Vitamin D Standardisation Program (VDSP) is a collaborative effort among the U.S. National Institutes of Health, Office of Dietary Supplements (NIH-ODS), the U.S. National Institute of Standards and Technology (NIST) the U.S. Centers for Disease Control and Prevention (CDC), national survey laboratories in several countries, and vitamin D researchers worldwide.

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3.3.1.2. Dose–response between vitamin D intake and serum 25(OH)D

Increasing oral vitamin D_2 and D_3 intake increases total 25(OH)D concentration until a plateau is reached after about 6 weeks, which indicates an equilibrium between the production, utilisation, storage, and degradation of serum 25(OH)D (Vieth, 1999; Viljakainen et al., 2006; Seamans and Cashman, 2009).

A linear relationship has been 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., 2011) and 50 μg/day (Cranney et al., 2007; EFSA NDA Panel, 2018). The IOM (2011) found a steeper rise in serum 25(OH)D concentrations with vitamin D intakes up to 25 μg/day and a slower, more flattened response when the intake was further increased. Similar results were obtained by Dunlop et al. (2021) in a meta-analysis including data from 34 RCTs on vitamin D fortification or biofortification in adults and children. Vitamin D_3 raised 25(OH)D concentrations more than vitamin D_2, with a threshold at ~ 26 nmol/L for a dose of ~ 21 μg/day.

A meta-regression analysis of the serum 25(OH)D response to total vitamin D intake in adults and children based on data collected through 35 trials (83 arms) was undertaken previously by EFSA (EFSA NDA Panel, 2016). The NDA Panel concluded that the non-linear model better described the dose–response curve. The main factors affecting the dose–response relationship were mean serum 25(OH)D concentration at baseline, geographical latitude, study start year, the analytical method used for measuring serum 25(OH)D and compliance (EFSA NDA Panel, 2016).

In supplementation trials conducted in adults 50 years of age or older using vitamin D (D_2 and D_3) at doses ranging from 5 to 250 μg/day (median, 20 μg/day), changes in serum 25(OH)D concentration for similar vitamin D doses could vary up to 3–4 times from trial to trial (Autier et al., 2012). The heterogeneity of the dose–response relationship between vitamin D intake and serum 25(OH)D concentrations has also been observed in more recent systematic reviews and meta-analysis of RCTs, in which baseline serum 25(OH)D concentration has been systematically identified as an important explanatory factor of such heterogeneity. Other factors include age, BMI, and dose administered (Mirhosseini et al., 2018; Dunlop et al., 2021; Cashman et al., 2022b; Nikooyeh et al., 2022).

At higher intakes, serum 25(OH)D concentration appears to be maintained within a narrow range < 75–220 nmol/L across vitamin D intakes from 20 μg to 250–500 μg/day, with a sharp rise in 25(OH)D concentrations with vitamin D intakes > 500 μg/day, suggesting a homeostatic regulatory mechanism with a buffer capacity that is exceeded at very high intakes (Vieth, 1999).

Several studies in adults have shown that vitamin D_2 supplements are less effective in raising or maintaining serum 25(OH)D concentrations compared to vitamin D_3 (Autier et al., 2012; Jones, 2013; Lehmann et al., 2013; Itkonen et al., 2016; Balachandar et al., 2021). Fortification of bread, biscuits or juice with either vitamin D_2 or vitamin D_3 increased serum 25(OH)D concentration in the same way as the corresponding supplement, but the increment was lower with vitamin D_2 than with vitamin D_3 (Natri et al., 2006; Tripkovic et al., 2017). In a systematic review based on 24 intervention studies, vitamin D_3 was found to be more efficient in increasing both total 25(OH)D and 25(OH)D_3 concentrations, and in regulating PTH concentrations, than vitamin D_2 irrespective of demographics, dosage and vehicle of supplementation (Balachandar et al., 2021). The authors acknowledge that, with doses typically used in fortified foods, vitamin D_3 may be only marginally better than vitamin D_2 for improving vitamin D status. The Panel notes that such difference was graphically presented and not quantified, and considers that the clinical relevance of the finding is unclear.

Available data suggest that vitamin D_3 may be the preferred substrate for hepatic conversion to 25 (OH)D (Holmberg et al., 1986; Tripkovic et al., 2012), and that vitamin D_3 and its metabolites have higher binding affinity to DBP as compared to vitamin D_2 (Houghton and Vieth, 2006). In addition, toxicity and repletion studies suggest some preferential non-specific catabolism of vitamin D_2 compared to vitamin D_3, accelerating its degradation, especially at 'high' doses (Jones, 2013; EFSA NDA Panel, 2018). No statistically significant differences between the effects of vitamin D_2 and vitamin D_3 intakes on serum total 25(OH)D concentrations have been observed among infants and young children, but data are limited and the sample size of the available studies is very small (Gordon et al., 2008; Gallo et al., 2013; EFSA NDA Panel, 2018).

It has been observed that vitamin D_2, either in fortified foods or supplements, decreases serum 25 (OH)D_3 concentrations to levels lower than those found in the placebo group, indicating possible replacement of serum 25(OH)D_3 by serum 25(OH)D_2 in the biological actions of vitamin D (Itkonen et al., 2016; Durrant et al., 2022). The long-term health effects of this remain unclear.

Compared to vitamin D_3, calcidiol monohydrate gives rise to a rapid and sustained increase in serum 25(OH)D concentrations (Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Navarro-Valverde

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et al., 2016; Vaes et al., 2018b; Graeff-Armas et al., 2020) (see Section 3.3.4) due to differences in the absorption pathway and the hydroxylation of native vitamin D_3 in the liver, which delays the increase in the serum 25(OH)D concentration of the vitamin as compared to calcidiol monohydrate (see Sections 3.2.2 and 3.2.6).

3.3.1.3. Factors affecting vitamin D status

Besides intake, the following factors have been identified as the main determinants of vitamin D status as assessed through serum 25(OH)D concentrations.

Sun exposure

Serum 25(OH)D concentrations vary according to season and latitude, with the lowest concentrations occurring at the end of winter and the highest concentrations at the end of summer (Engelsen, 2010; Webb et al., 2021), generally reflecting the amount of endogenous synthesis following UV-B radiation. Sun exposure is also affected by individual behavioural factors, like time spent outdoors, clothing, use of sunscreens, etc. Data on both season and latitude, where available, have been extracted from the studies included in this opinion.

Ethnicity and skin type

Pigmentation and thickness of the skin, which are largely genetically determined (Bouillon, 2017), have a major impact on the dermal synthesis of vitamin D_3, although cultural habits affecting exposure to available UV-B irradiation such lifestyle, outdoor activities, and clothing, may also contribute to differences in serum 25(OH)D concentrations among ethnic groups. Adults with Fitzpatrick skin type V (brown) may need a dose of simulated sunlight that is 2.5–3 times that required by white Caucasians to raise circulating 25(OH)D to the same extent (Farrar et al., 2013; Webb et al., 2018).

The highest risk of dark-skinned ethnic subgroups for low vitamin D status at high latitudes has been systematically documented (Cashman et al., 2015; Cashman et al., 2016; Herrick et al., 2019; Hastie et al., 2020; Darling et al., 2021; Cashman et al., 2022a). Data on both ethnicity and baseline serum 25(OH)D concentrations, where available, have been extracted from the studies included in this opinion.

Age

Lower 25(OH)D concentrations have been generally reported in older adults as compared to young individuals. Lower dietary intakes, less exposure to sunlight, lower efficiency of vitamin D synthesis in the skin and possibly lower hydroxylation of vitamin D in the liver may contribute to this phenomenon, whereas the intestinal absorption of vitamin D does not appear to be affected by age (Borel et al., 2015).

BMI and adiposity

An inverse relationship between BMI/total body fat and serum 25(OH)D concentrations has been reported in several studies (Saneei et al., 2013; Vanlint, 2013; Rejnmark et al., 2017). A recent genome-wide association study (GWAS) provides strong support for the hypothesis that high BMI is causal (directly or indirectly) for low serum 25(OH)D, and not the opposite (Revez et al., 2020). Possible mechanisms mediating this phenomenon include sequestration of vitamin D mostly in adipose tissue, a volumetric dilution of vitamin D, and behavioural factors (e.g. body hiding) leading to lower cutaneous vitamin D synthesis (EFSA NDA Panel, 2016). Surrogate measures of central adiposity (e.g. waist circumference, waist-to-hip ratio, trunk fat by DXA) have also been associated with lower serum 25(OH)D concentrations and higher risk of vitamin D deficiency in cross-sectional studies (Snijder et al., 2006; Ding et al., 2010; Zhang et al., 2015).

The higher vitamin D stores in adipose tissue, and particularly in visceral fat, of overweight and obese individuals as compared to normal-weight subjects has several implications. First, weight loss through diet or gastric surgery (5% to $>$ 10% body weight) significantly increases serum 25(OH)D concentrations and improves vitamin D status (Himbert et al., 2017). In a randomised lifestyle intervention trial in men with central obesity, a 50% reduction in the volume of visceral adipose tissue (assessed by computed tomography) was associated with a concomitant 26% increase in serum 25 (OH)D concentration (Gangloff et al., 2015). Second, higher doses of vitamin D (about 2–3 times higher, depending on the study) are needed to reach target serum 25(OH)D concentrations in obese adults, children and adolescents than in their lean counterparts (Hypponen and Boucher, 2018). This may explain why BMI is an important factor contributing to the heterogeneity in the biological

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response to vitamin D supplementation across trials (Hypponen and Boucher, 2018). For these reasons, data on BMI for the study population, where available, have been extracted from the studies included in this opinion.

Genetic polymorphisms

Some polymorphisms of genes encoding proteins involved in vitamin D synthesis, transport and metabolism influence serum 25(OH)D concentration (Berry and Hyppönen, 2011). GWAS on data from subjects of European ancestry (Ahn et al., 2010; Wang et al., 2010; Jiang et al., 2018) have identified four common single-nucleotide polymorphisms that, combined, explain from 5% to 7.5% of the variation in serum 25(OH)D: *GC* (group specific component gene), *DHCR7*, *CYP2R1*, and *CYP24A1*, expressing DBP, delta-7-dehydrocholesterol reductase (DHCR7), 25-hydroxylase and 24-hydroxylase. A latest GWAS on data from the UK-Biobank and including 417,580 Europeans has identified 143 independent loci in 112 1-Mb regions associated with serum 25(OH)D concentration, implicating genes involved in lipid and lipoprotein metabolism, dermal tissue properties, and the sulfation and glucuronidation of 25(OH)D (Revez et al., 2020). Twin and family studies have led to a wide range of heritability estimates (from 0% to 90%) for serum 25(OH)D. From twin studies, the genetic influence on the serum concentration of 25(OH)D has been estimated to explain about 50% of its variation (Bouillon and Carmeliet, 2018), half of which may be related to skin colour and sun exposure behaviour (Mitchell et al., 2019).

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, the substrate for the 25(OH)D synthesis in the skin, leading to higher vitamin D status (EFSA NDA Panel, 2016). Variants in *GC* and *CYP2R1* have been associated with lower serum 25(OH)D, whereas inactivating mutations in the gene encoding CYP24A1, which catalyses the conversion of both $25(OH)D_3$ and $1,25(OH)_2D_3$ into 24-hydroxylated products for excretion, causes idiopathic infantile hypercalcaemia, a condition leading to nephrolithiasis and nephrocalcinosis (Jones et al., 2012; Jones et al., 2017), with an estimated frequency in Poland of 1:32,465 births (Pronicka et al., 2017). Significant associations between *CYP27B1* (coding for 1α-hydroxylase) or *VDR* genotypes and serum 25(OH)D concentrations have not been consistently found (EFSA NDA Panel, 2016).

Genetic polymorphisms affecting serum 25(OH) concentrations have been studied in relation to the response to vitamin D supplementation. In a Danish study (Nissen et al., 2015), common mutations in the *CYP2R1* and *GC* showed the lowest increase in serum 25(OH)D after whole-body UV-B radiation or consumption of vitamin D_3–fortified bread and milk, the effect being comparable for both treatments. Mutations in these two genes were also significantly associated with variations in serum 25(OH)D concentrations and efficacy of response to vitamin D supplementation in Tunisian adults (Ammar et al., 2022). In a Chinese study (Yao et al., 2017), genetic factors (mutations in the *GS*, *VDR* and *CYP2R1* genes) showed a larger impact on serum 25(OH)D after supplementation with 50 μg/day vitamin D_3 than non-genetic factors including baseline value, BMI, and sex. Sex was not significantly associated with serum 25(OH)D in the GWAS by Revez et al. (2020).

Despite the progress made in understanding the effect of genotype on vitamin D synthesis, transport, metabolism and response to supplementation, the Panel considers that data available are insufficient for use in establishing a UL for vitamin D according to genotype variants.

3.3.2. Serum parathyroid hormone concentration

Parathyroid glands secrete PTH in response to low serum calcium levels to maintain serum calcium concentration within a narrow range through its action on the kidneys, bone, and small intestine. This mechanism is subject to negative-feedback loop. PTH also facilitates the synthesis of $1,25(OH)_2D_3$ in the kidneys (see Section 3.2.6), and together with this active form of vitamin D regulates calcium and phosphate in blood (Khan et al., 2022).

Serum PTH has been suggested as a possible biomarker or functional endpoint of vitamin D status. Vitamin D supplementation may suppress serum PTH directly because $1,25(OH)_2D_3$ downregulates transcription of PTH and expression of the calcium-sensing receptor, and indirectly through its effects on intestinal calcium absorption. Data from observational studies, however, do not allow setting a cut-off value for 25(OH)D concentration using PTH as a reference, and RCTs show a wide and heterogeneous response of PTH to vitamin D supplementation, particularly in co-supplementation with calcium (EFSA NDA Panel, 2016).

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In a recent cross-sectional analysis of data from a general health survey in Norway, a gradual decrease in serum PTH with increasing serum 25(OH)D with no apparent plateau was observed in females, whereas the decrease in PTH in subjects with serum 25(OH)D > 74 nmol/L was marginal in males. In a pooled analysis of data from five RCTs on vitamin D supplementation including individuals with high levels of 25(OH)D at baseline, serum PTH suppression by vitamin D appeared to reach its full effect after 3 months and, as expected, was higher in subjects with lower 25(OH)D concentration showing highest PTH values at baseline. PTH suppression following vitamin D supplementation, however, was statistically significant across the whole range of baseline 25(OH)D (from < 25 nmol/L to > 100 nmol/L) and remained so even after adjusting for changes in serum calcium, suggesting a direct effect on PTH synthesis/secretion (Jorde and Grimnes, 2020).

The Panel notes that serum PTH cannot be used as a marker of vitamin D intake or status. However, serum PTH concentrations were extracted from RCTs comparing the effects of supplementation with calcidiol monohydrate versus vitamin D_3 on serum 25(OH)D as complementary information (see Section 3.3.4).

3.3.3. Other biomarkers

The fraction of 25(OH)D that is not bound to DBP or albumin represents < 1% of the total pool, but it is readily available to target cells for conversion into the metabolically active 1,25 $(OH)_2D$ by 1α-hydroxylation. Its potential as a biomarker for vitamin D status remains to be established (EFSA NDA Panel, 2016).

1,25$(OH)_2D$ has a very short half-life (hours) and its serum concentration (about 1,000 times lower than that of 25(OH)D) is tightly regulated (Lips, 2007). Whereas 1,25$(OH)_2D$ reflects vitamin D function and correlates with blood calcium, phosphorous and PTH concentrations, it cannot be used as a marker of vitamin D intake or status (EFSA NDA Panel, 2016).

Other biomarkers that reflect one or more functions of vitamin D have been proposed, either alone or in combination, to assess vitamin D deficiency, including markers of bone turn-over, bone structure and bone mineralization, and calcium/phosphorous concentrations in blood or urine. However, none of these can be considered as reliable markers of vitamin D intake or status (EFSA NDA Panel, 2016).

3.3.4. Conversion factor for calcidiol monohydrate into vitamin D_3

Details on the systematic review that was conducted to address this sub-question can be found in Section 2.1 of this opinion and section 2.3 of the external technical report (Lamberg-Allardt et al., 2023). A detailed description of the methods used for data preparation, plotting and analyses is available in the statistical report (Annex F of the external technical report).

3.3.4.1. Body of evidence

The BoE consists of 12 RCTs comparing the effects of supplementation with calcidiol monohydrate versus vitamin D_3 on serum 25(OH)D concentrations meeting the eligibility criteria for the purpose of deriving a conversion factor for calcidiol monohydrate (see Section 2.1.1). The characteristics and results of these studies can be found in the evidence table (Appendix C.1). Of these, 9 also reported on serum PTH concentrations.

The heatmap for the RoB assessment is in Appendix B.1. Six RCTs were in tier 1, two in tier 2 and four in tier 3. Critical domains were exposure characterisation (n = 5, due to the fact that the vitamin D dose in the supplements was not analysed and/or compliance was not reported), outcome assessment (n = 6; mostly because of the method used and/or lack of information about its performance or the blinding of outcome assessors), allocation concealment (n = 7; mostly not reported), and blinding (n = 6).

Two RCTs could not be used for evidence synthesis. One RCT (Ruggiero et al., 2019) conducted in Italy provided supplements containing 150 μg/week (21.43 μg/day) of calcidiol monohydrate or vitamin D_3, both with calcium (1 g/day), for 7 months to community-dwelling men and women > 75 years of age. Serum 25(OH)D concentrations increased by an average of 47.5 nmol/L in the calcidiol group and by 40 nmol/L in the vitamin D_3 group (p = 0.5). No dispersion statistics or an exact p value for changes within groups were provided in the publication, and thus the study was not considered for data analysis in relation to this variable but was kept for assessing the effects of calcidiol versus vitamin D_3 on serum PTH. In the second RCT (Jakobsen et al., 2017), young male adults living in Denmark consumed 10 μg/day of vitamin D_2, vitamin D_3 or calcidiol for 6 weeks each following a randomised cross-over design, with no washout period. Mean achieved serum 25(OH)D

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concentrations were approximately 10 nmol/L higher with calcidiol compared to vitamin D_3 (63.8 nmol/L [95% CI: 59.9, 67.9] vs. 54.4 nmol/L [95% CI: 51.1, 58.0 nmol/L], respectively; $p < 0.001$). The study also assessed serum PTH. Owing to possible carry-over effects not addressed in the publication and the use of vitamin D_2, the study was not used for any data analysis. The Panel notes, however, that calcidiol increased serum 25(OH)D concentrations significantly more than vitamin D_3 when both were given at the same doses of ~ 21 and 10 μg/day in these studies.

The characteristics of the 10 RCTs available for data analysis on serum 25(OH)D concentrations are briefly summarised below.

Nine RCTs were conducted in Europe (Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Catalano et al., 2015; Navarro-Valverde et al., 2016; Vaes et al., 2018a,b; Graeff-Armas et al., 2020; Corrado et al., 2021; Okoye et al., 2022), and thus in Caucasian or assumed Caucasian populations, and one in the USA (Shieh et al., 2017) in a population of mixed ethnic origin (Appendix C.1). Except for the study run in the USA (age ≥ 18 years; sex not specified), RCTs were in adults of both sexes (n = 5) or females only (n = 4) 50 years of age and older. Whereas most RCTs were in healthy free-living adults, in four RCTs the study population was recruited at the hospital among females with osteopenia (Catalano et al., 2015), osteoporosis (Navarro-Valverde et al., 2016) or hypovitaminosis D (Corrado et al., 2021), or among geriatric patients consecutively hospitalised for acute illness (Okoye et al., 2022).

Doses of vitamin D ranged from 5 to 38 μg/day as calcidiol and from 20 to 62.5 μg/day as vitamin D_3. The same doses of calcidiol and vitamin D_3 were used in six RCTs (20 μg/day in five and 25 μg/day in one). Four of these RCTs had multiple calcidiol arms. In the remaining four RCTs, doses of calcidiol were always lower than reference doses of vitamin D_3 (see Figure 2). In the three RCTs that verified the content of vitamin D supplements (Vaes et al., 2018a; Vaes et al., 2018b; Graeff-Armas et al., 2020), the analysed doses were very close to labelled doses, and thus the latter was used for further analyses.

Vitamin D was consumed only daily in 5 studies (Cashman et al., 2012; Shieh et al., 2017; Vaes et al., 2018a,b; Graeff-Armas et al., 2020) and only weekly in 3 studies (Catalano et al., 2015; Corrado et al., 2021; Okoye et al., 2022). Weekly doses were transformed in daily doses for data analysis (Figure 2). In one RCT (Bischoff-Ferrari et al., 2012) where the same doses of calcidiol and vitamin D were provided both daily and weekly (four intervention arms), achieved serum 25(OH)D concentrations did not differ significantly between weekly and daily supplementation, and thus the results for the two calcidiol arms and for the two vitamin D_3 arms were combined by the authors. In another RCT (Navarro-Valverde et al., 2016), vitamin D_3 was provided daily and calcidiol daily, weekly, and every other week (total of 4 arms). Data has been extracted for arms giving calcidiol daily (20 μg/day, as for vitamin D_3) and weekly (266 μg/week, corresponding to 38 μg/day). Calcium was not given as co-supplementation in any of the 10 RCTs.

The duration of the intervention ranged from 2.5 to 6 months, which ensures stable serum 25(OH)D concentrations following supplementation. Since the achieved serum 25(OH)D concentrations at 6 months and 12 months were not significantly different for the only RCT that lasted 12 months (Navarro-Valverde et al., 2016), data at 6 months was used for analysis to increase comparability across studies.

Mean baseline serum 25(OH)D concentrations ranged from 30.7 nmol/L to 55.8 nmol/L across the intervention arms and were > 50 nmol/L in one RCT only (Catalano et al., 2015). Six RCTs measured serum 25(OH)D concentrations using HPLC–MS/MS, LC–MS/MS, or HPLC + UV assays, two reported on the use of CLIA (Shieh et al., 2017; Corrado et al., 2021), one the use of ELISA (Cashman et al., 2012), and one did not report which assay was used (Catalano et al., 2015).

Seven of the 10 RCTs available for data analysis on serum 25(OH)D concentrations also measured serum PTH concentrations (Bischoff-Ferrari et al., 2012; Cashman et al., 2012; Navarro-Valverde et al., 2016; Vaes et al., 2018a,b; Ruggiero et al., 2019; Graeff-Armas et al., 2020), but one (Corrado et al., 2021) did not report the units of measurement and could not be included in data analysis (Figure 4).

3.3.4.2. Effect of calcidiol monohydrate versus vitamin D_3 on serum 25(OH)D concentrations

The achieved (end-of-trial) serum 25(OH)D concentrations were higher with calcidiol than with vitamin D_3, except when calcidiol doses were much lower (i.e., 5 and 7 μg/day) than vitamin D_3 doses (i.e., 20 μg/day) (see mean differences in Figure 2a). In the four RCTs examining the dose–response of calcidiol compared to a single dose of vitamin D_3 (Cashman et al., 2012; Navarro-Valverde

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et al., 2016; Vaes et al., 2018b; Graeff-Armas et al., 2020), the achieved serum 25(OH)D concentrations (absolute values) were higher for calcidiol as compared to vitamin D_3 and increased with increasing doses of calcidiol.

Owing to the disparity of vitamin D_3 and calcidiol doses used within and across studies, the mean difference in the achieved serum 25(OH)D concentrations per μg/day between calcidiol and vitamin D_3 (reference) was calculated. In all RCTs, the achieved serum 25(OH)D concentrations per μg/day of vitamin D were higher with calcidiol than with vitamin D_3, although mean differences between calcidiol and vitamin D_3 tended to decrease with increasing doses of calcidiol in the four RCTs that assessed multiple calcidiol doses (Figure 2b).

To assess the relative bioavailability of calcidiol versus vitamin D_3, the ratio of mean achieved serum 25(OH)D concentrations per μg/day between calcidiol and vitamin D_3 (i.e., ratio of means (ROM)) was calculated within each study for all calcidiol arms (Figure 2c). The mean ratio ranged from 1.31 to 4.62 across studies, depending on the dose of calcidiol tested but also on the dose of vitamin D_3 used as reference. When 20 μg/day of vitamin D_3 were used as reference (n = 7 RCTs), the mean ratio ranged from 1.40 to 2.93 for doses of calcidiol ranging from 5 to 38 μg/day, the ratio decreasing with increasing doses of calcidiol. However, when much higher doses of vitamin D_3 were used as reference (~ 60 μg/day) the mean ratio was 4.30 to 4.62 for calcidiol doses of 20 μg/day (n = 2 RCTs). This could be explained by the flattening of the linear dose–response between vitamin D_3 intake and serum 25(OH)D at intakes above 30–50 μg/day (see Section 3.3.1.2).

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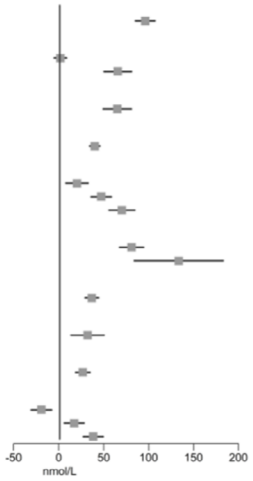
(a)

Publication	Country	Duration	Sex	Age	Group	Dose, µg/d	N Analysed	Mean S-25(OH)D: Baseline, nmol/L	Mean S-25(OH)D: Achieved, nmol/L	MD [95%-CI]	Assay	RoB Tier
Bischoff-Ferrari et al., 2012	CH	4 mo	F	50–70	D3	20	10	35.5	77.5		HPLC-MS/MS	1
Bischoff-Ferrari et al., 2012	CH	4 mo	F	50–70	25(OH)D3	20	10	30.7	173.7	96.2 [85.2; 107.2]	HPLC-MS/MS	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	D3	20	13	49.7	69.0		ELISA	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	25(OH)D3	7	14	42.5	70.7	1.7 [-5.3; 8.7]	ELISA	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	25(OH)D3	20	12	38.2	134.6	65.6 [50.1; 81.1]	ELISA	1
Catalano et al., 2015	IT	24 wk	F	59.0 [mean]	D3	20	28	50.9	60.8		NR	3
Catalano et al., 2015	IT	24 wk	F	59.0 [mean]	25(OH)D3	20	29	55.8	125.8	65.1 [49.3; 80.9]	NR	3
Corrado et al., 2021	IT	6 mo	F	60.8 [mean]	D3	25	27	31.3	127.3		CLIA	2
Corrado et al., 2021	IT	6 mo	F	60.8 [mean]	25(OH)D3	25	26	33.3	167.3	40.0 [34.3; 45.7]	CLIA	2
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	D3	20	21	47.1	82.8		LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	10	21	48.0	103.0	20.2 [7.7; 32.7]	LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	15	23	49.5	130.0	47.2 [35.8; 58.6]	LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	20	24	48.5	153.0	70.2 [55.7; 84.7]	LC-MS/MS	1
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	D3	20	10	40.5	80		HPLC + UV	3
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	25(OH)D3	20	10	37.2	161.0	81.0 [67.5; 94.5]	HPLC + UV	3
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	25(OH)D3	38	10	38.0	213.5	133.5 [83.9; 183.1]	HPLC + UV	3
Okoye et al., 2022	IT	3 mo*	FM	83 [mean]	D3	62.5	69	42.0	76.75		HPLC-MS/MS	3
Okoye et al., 2022	IT	3 mo*	FM	83 [mean]	25(OH)D3	20	71	47.0	113.5	36.8 [29.2; 44.3]	HPLC-MS/MS	3
Shieh et al., 2017	US	16 wk	NR	≥ 18	D3	60	16	40.5	74.0		CLIA	1
Shieh et al., 2017	US	16 wk	NR	≥ 18	25(OH)D3	20	19	42.5	106.0	32.0 [13.4; 50.6]	CLIA	1
Vaes et al., 2018a	NL	6 mo	FM	≥ 65	D3	20	24	36.3	72.0		LC-MS/MS	1
Vaes et al., 2018a	NL	6 mo	FM	≥ 65	25(OH)D3	10	26	38.1	98.7	26.7 [18.6; 34.8]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	D3	20	11	37.7	71.6		LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	5	12	43.4	52.2	-19.4 [-30.9; -7.9]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	10	14	38.3	88.7	17.1 [5.9; 28.3]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	15	14	38.6	109.9	38.3 [27.1; 49.5]	LC-MS/MS	1

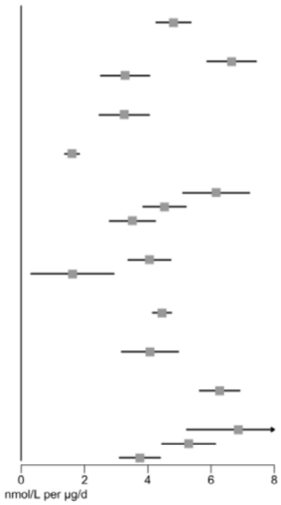
Mean difference in achieved S-25(OH)D: 25(OH)D3 vs vitamin D3



(b)

Publication	Country	Duration	Sex	Age	Group	Dose, µg/d	N Analysed	Achieved S-25(OH)D, nmol/L per µg/d of vitamin D	MD [95%-CI]	Assay	RoB Tier
Bischoff-Ferrari et al., 2012	CH	4 mo	F	50–70	D3	20	10	3.9		HPLC-MS/MS	1
Bischoff-Ferrari et al., 2012	CH	4 mo	F	50–70	25(OH)D3	20	10	8.7	4.8 [4.3; 5.4]	HPLC-MS/MS	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	D3	20	13	3.5		ELISA	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	25(OH)D3	7	14	10.1	6.6 [5.9; 7.4]	ELISA	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	25(OH)D3	20	12	6.7	3.3 [2.5; 4.1]	ELISA	1
Catalano et al., 2015	IT	24 wk	F	59.0 [mean]	D3	20	28	3.0		NR	3
Catalano et al., 2015	IT	24 wk	F	59.0 [mean]	25(OH)D3	20	29	6.3	3.3 [2.5; 4.0]	NR	3
Corrado et al., 2021	IT	6 mo	F	60.8 [mean]	D3	25	27	5.1		CLIA	2
Corrado et al., 2021	IT	6 mo	F	60.8 [mean]	25(OH)D3	25	26	6.7	1.6 [1.4; 1.8]	CLIA	2
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	D3	20	21	4.1		LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	10	21	10.3	6.2 [5.1; 7.2]	LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	15	23	8.7	4.5 [3.8; 5.2]	LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	20	24	7.7	3.5 [2.8; 4.2]	LC-MS/MS	1
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	D3	20	10	4.0		HPLC + UV	3
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	25(OH)D3	20	10	8.1	4.0 [3.4; 4.7]	HPLC + UV	3
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	25(OH)D3	38	10	5.6	1.6 [0.3; 2.9]	HPLC + UV	3
Okoye et al., 2022	IT	3 mo*	FM	83 [mean]	D3	62.5	69	1.2		HPLC-MS/MS	3
Okoye et al., 2022	IT	3 mo*	FM	83 [mean]	25(OH)D3	20	71	5.7	4.4 [4.2; 4.7]	HPLC-MS/MS	3
Shieh et al., 2017	US	16 wk	NR	≥ 18	D3	60	16	1.2		CLIA	1
Shieh et al., 2017	US	16 wk	NR	≥ 18	25(OH)D3	20	19	5.3	4.1 [3.2; 5.0]	CLIA	1
Vaes et al., 2018a	NL	6 mo	FM	≥ 65	D3	20	24	3.6		LC-MS/MS	1
Vaes et al., 2018a	NL	6 mo	FM	≥ 65	25(OH)D3	10	26	9.9	6.3 [5.6; 6.9]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	D3	20	11	3.6		LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	5	12	10.4	6.9 [5.2; 8.5]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	10	14	8.9	5.3 [4.4; 6.1]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	15	14	7.3	3.7 [3.1; 4.4]	LC-MS/MS	1

Mean difference in achieved S-25(OH)D per µg/d of vitamin D: 25(OH)D3 vs vitamin D3



(c)

Publication	Country	Duration	Sex	Age	Group	Dose, µg/d	N Analysed	Achieved S-25(OH)D, nmol/L per µg/d of vitamin D	ROM [95%-CI]	Assay	RoB Tier
Bischoff-Ferrari et al., 2012	CH	4 mo	F	50–70	D3	20	10	3.9		HPLC-MS/MS	1
Bischoff-Ferrari et al., 2012	CH	4 mo	F	50–70	25(OH)D3	20	10	8.7	2.2 [2.0; 2.5]	HPLC-MS/MS	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	D3	20	13	3.5		ELISA	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	25(OH)D3	7	14	10.1	2.9 [2.6; 3.2]	ELISA	1
Cashman et al., 2012	IE	10 wk	FM	≥ 50	25(OH)D3	20	12	6.7	2.0 [1.7; 2.2]	ELISA	1
Catalano et al., 2015	IT	24 wk	F	59.0 [mean]	D3	20	28	3.0		NR	3
Catalano et al., 2015	IT	24 wk	F	59.0 [mean]	25(OH)D3	20	29	6.3	2.1 [1.8; 2.4]	NR	3
Corrado et al., 2021	IT	6 mo	F	60.8 [mean]	D3	25	27	5.1		CLIA	2
Corrado et al., 2021	IT	6 mo	F	60.8 [mean]	25(OH)D3	25	26	6.7	1.3 [1.3; 1.4]	CLIA	2
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	D3	20	21	4.1		LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	10	21	10.3	2.5 [2.2; 2.8]	LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	15	23	8.7	2.1 [1.9; 2.3]	LC-MS/MS	1
Graeff-Armas et al., 2020	UK	6 mo	FM	> 50	25(OH)D3	20	24	7.7	1.8 [1.6; 2.1]	LC-MS/MS	1
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	D3	20	10	4.0		HPLC + UV	3
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	25(OH)D3	20	10	8.1	2.0 [1.8; 2.2]	HPLC + UV	3
Navarro-Valverde et al., 2016	ES	6 mo	F	67 [mean]	25(OH)D3	38	10	5.6	1.4 [1.1; 1.8]	HPLC + UV	3
Okoye et al., 2022	IT	3 mo*	FM	83 [mean]	D3	62.5	69	1.2		HPLC-MS/MS	3
Okoye et al., 2022	IT	3 mo*	FM	83 [mean]	25(OH)D3	20	71	5.7	4.6 [4.3; 5.0]	HPLC-MS/MS	3
Shieh et al., 2017	US	16 wk	NR	≥ 18	D3	60	16	1.2		CLIA	1
Shieh et al., 2017	US	16 wk	NR	≥ 18	25(OH)D3	20	19	5.3	4.3 [3.6; 5.2]	CLIA	1
Vaes et al., 2018a	NL	6 mo	FM	≥ 65	D3	20	24	3.6		LC-MS/MS	1
Vaes et al., 2018a	NL	6 mo	FM	≥ 65	25(OH)D3	10	26	9.9	2.7 [2.5; 3.0]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	D3	20	11	3.6		LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	5	12	10.4	2.9 [2.4; 3.5]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	10	14	8.9	2.5 [2.1; 2.9]	LC-MS/MS	1
Vaes et al., 2018b	NL	24 wk	FM	≥ 65	25(OH)D3	15	14	7.3	2.0 [1.8; 2.3]	LC-MS/MS	1

ROM of achieved S-25(OH)D per µg/d of vitamin D: 25(OH)D3 vs vitamin D3

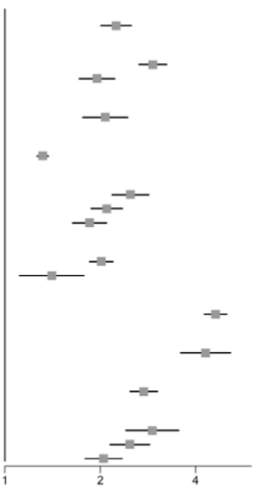


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Figure 2: The effect of calcidiol monohydrate on serum 25(OH)D concentration compared to vitamin D_3: (a) difference of means of achieved serum 25(OH)D concentration; (b) difference of means of achieved serum 25(OH)D concentration per μg/day of vitamin D; (c) ratio of means (ROM) of achieved serum 25(OH)D concentration per μg/day of vitamin D

Duration of the intervention as reported by the authors. For age, recruitment target range is presented unless otherwise indicated. Achieved concentration refers to serum 25(OH)D concentration at the end of the treatment. Mean difference = mean serum 25(OH)D concentrations achieved with (a) calcidiol minus those achieved with vitamin D_3, (b) per μg/day of vitamin D, and (c) ratio of means. **Abbreviations:** CI, confidence interval; CH, Switzerland; CLIA, chemiluminescence immune assay; D_3, vitamin D_3; ELISA, enzyme-linked immunosorbent assay; ES, Spain; F, females; HPLC–MS/MS, high-performance liquid chromatography tandem mass spectrometry; IE, Ireland; IT, Italy; LC–MS/MS, liquid chromatography tandem mass spectrometry; M, males; MD, mean difference; NL, Netherlands; NR, not reported; RoB, risk of bias; ROM = ratio of means; S-25(OH)D, serum 25-hydroxyvitamin D; UK, United Kingdom; UV, ultraviolet; US, United States; 25(OH)D_3, 25-hydroxyvitamin D_3, i.e., calcidiol. *From hospital admission to 3 months after discharge.

To control for the possible confounding introduced by differences in the dose of calcidiol and vitamin D_3 when investigating their relative bioavailability, the six RCTs that used the same doses were selected and meta-analysed using 25(OH)D concentrations as the outcome variable. Five RCTs used 20 μg/day and one used 25 μg/day (Figure 3a). The effect size was the ratio of mean (RoM) achieved serum 25(OH)D concentrations per μg/day of the vitamin D form administered. The random-effects model was used applying the inverse-variance method to pool the estimates using the DerSimonian-Laird approach (DerSimonian and Laird, 1986). The pooled ROM (95% CI) was 1.87 (1.53, 2.29). The model indicated significant heterogeneity (I^2 = 97.1%, 95% CI = 95.5–98.2%).

Outliers were defined as studies for which the 95% CI was falling completely outside the pooled effect 95% CI. Therefore, the RCT using a dose of 25 μg/day for both vitamin D_3 and calcidiol (Corrado et al., 2021) was identified as an outlier (95% CI of the study: 1.26, 1.37; 95% CI of the pooled estimate: 1.53, 2.29), in line with the BoE indicating that the achieved serum 25(OH)D concentrations per μg/day of vitamin D decreased with increasing doses of calcidiol as compared to vitamin D_3. Omitting this study from the model increased the pooled estimate and reduced sampling uncertainty: the ROM (95% CI) was 2.02 (1.85, 2.21). Heterogeneity was also reduced significantly (I^2 = 31.2%, 95% CI = 0.0–73.6%). However, the CI of the pooled estimate without the study overlaps with that obtained including the study, and none of the studies was found to be influential based on leave out analysis.

A meta-analysis was also performed using the ROM as the measure of effect while including all RCTs and all calcidiol arms (Figure 3b). Therefore, each ROM might compare different doses of calcidiol and vitamin D_3. This potential confounding factor is only partially controlled by the normalisation of the effect by unit dose. The hierarchical structure in the data was reflected in the random components (arms nested within studies). In the random effects model, the pooled ROM (95% CI) was 2.40 (1.89, 3.06). The model indicated high heterogeneity (I^2 = 98.5%, 95% CI = 98.2–98.8%). No studies were identified as being influential based on leave out analysis. Excluding the three studies in RoB Tier 3 (Catalano et al., 2015; Navarro-Valverde et al., 2016; Okoye et al., 2022) had little impact on the results: ROM (95% CI) was 2.36 (1.82, 3.10) and heterogeneity remained high (Figure 3c).

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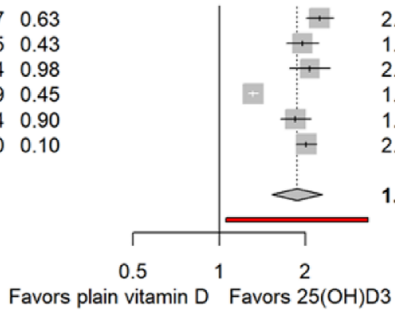
(a)

Study	25(OH)D3 per µg dose	n	Mean	SD	Vit-D3 per µg n	Mean	SD	Ratio of Means	ROM	95%-CI	Weight
Bischoff-Ferrari et al., 2012	20	10	8.68	0.62	10	3.87	0.63		2.24	[2.01; 2.50]	16.7%
Cashman et al., 2012	20	12	6.73	1.30	13	3.45	0.43		1.95	[1.71; 2.22]	16.3%
Catalano et al., 2015	20	29	6.29	1.93	28	3.04	0.98		2.07	[1.76; 2.44]	15.3%
Corrado et al., 2021	25	26	6.69	0.40	27	5.09	0.45		1.31	[1.26; 1.37]	18.0%
Graeff-Armas et al., 2020	20	24	7.65	1.53	21	4.14	0.90		1.85	[1.63; 2.09]	16.4%
Navarro-Valverde et al., 2016	20	10	8.05	1.08	10	4.00	0.10		2.01	[1.85; 2.19]	17.3%
Random effects model		**111**			**109**				**1.87**	**[1.53; 2.29]**	**100.0%**
Prediction interval										**[1.06; 3.31]**	

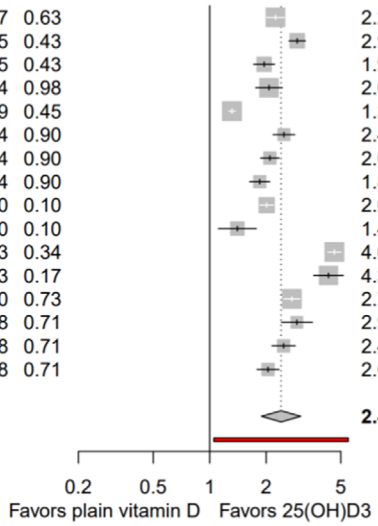
Heterogeneity: I^2 = 97%, τ^2 = 0.036, p < 0.01



(b)

Study	n	25(OH)D3 per µg dose	Mean	SD	n	Vit−D3 per µg dose	Mean	SD	Ratio of Means	ROM	95%−CI	Weight
Bischoff−Ferrari et al., 2012	10	20	8.68	0.62	10	20	3.87	0.63		2.24	[2.01; 2.50]	9.2%
Cashman et al., 2012	14	7	10.10	1.41	13	20	3.45	0.43		2.93	[2.65; 3.24]	5.6%
Cashman et al., 2012	12	20	6.73	1.30	13	20	3.45	0.43		1.95	[1.71; 2.22]	5.4%
Catalano et al., 2015	29	20	6.29	1.93	28	20	3.04	0.98		2.07	[1.76; 2.44]	9.0%
Corrado et al., 2021	26	25	6.69	0.40	27	25	5.09	0.45		1.31	[1.26; 1.37]	9.4%
Graeff−Armas et al., 2020	21	10	10.30	2.29	21	20	4.14	0.90		2.49	[2.18; 2.84]	3.8%
Graeff−Armas et al., 2020	23	15	8.67	1.37	21	20	4.14	0.90		2.09	[1.87; 2.35]	3.9%
Graeff−Armas et al., 2020	24	20	7.65	1.53	21	20	4.14	0.90		1.85	[1.63; 2.09]	3.9%
Navarro−Valverde et al., 2016	10	20	8.05	1.08	10	20	4.00	0.10		2.01	[1.85; 2.19]	6.1%
Navarro−Valverde et al., 2016	10	38	5.62	2.11	10	20	4.00	0.10		1.40	[1.11; 1.77]	4.7%
Okoye et al., 2022	71	20	5.67	1.23	69	62	1.23	0.34		4.62	[4.26; 5.02]	9.3%
Shieh et al., 2017	19	20	5.30	1.99	16	60	1.23	0.17		4.30	[3.58; 5.15]	8.9%
Vaes et al., 2018a	26	10	9.87	1.47	24	20	3.60	0.73		2.74	[2.48; 3.03]	9.3%
Vaes et al., 2018b	12	5	10.44	2.79	11	20	3.58	0.71		2.92	[2.41; 3.53]	3.6%
Vaes et al., 2018b	14	10	8.87	1.40	11	20	3.58	0.71		2.48	[2.15; 2.86]	3.9%
Vaes et al., 2018b	14	15	7.33	0.94	11	20	3.58	0.71		2.05	[1.79; 2.34]	4.0%
Random effects model	**335**				**316**					**2.40**	**[1.89; 3.06]**	**100.0%**
Prediction interval											**[1.05; 5.48]**	

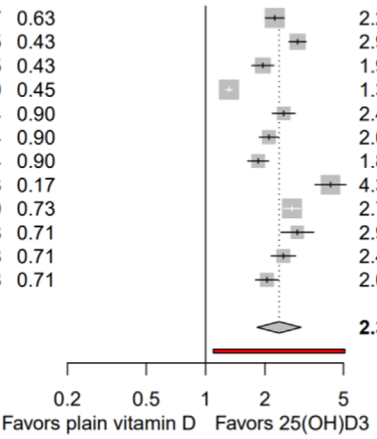
Heterogeneity: I^2 = 99%, τ^2 = 0.135, p < 0.01



(c)

Study	n	25(OH)D3 per mcg dose	Mean	SD	n	Vit−D3 per mcg dose	Mean	SD	Ratio of Means	ROM	95%−CI	Weight
Bischoff−Ferrari et al., 2012	10	20	8.68	0.62	10	20	3.87	0.63		2.24	[2.01; 2.50]	12.6%
Cashman et al., 2012	14	7	10.10	1.41	13	20	3.45	0.43		2.93	[2.65; 3.24]	8.0%
Cashman et al., 2012	12	20	6.73	1.30	13	20	3.45	0.43		1.95	[1.71; 2.22]	7.7%
Corrado et al., 2021	26	25	6.69	0.40	27	25	5.09	0.45		1.31	[1.26; 1.37]	12.9%
Graeff−Armas et al., 2020	21	10	10.30	2.29	21	20	4.14	0.90		2.49	[2.18; 2.84]	5.6%
Graeff−Armas et al., 2020	23	15	8.67	1.37	21	20	4.14	0.90		2.09	[1.87; 2.35]	5.8%
Graeff−Armas et al., 2020	24	20	7.65	1.53	21	20	4.14	0.90		1.85	[1.63; 2.09]	5.7%
Shieh et al., 2017	19	20	5.30	1.99	16	60	1.23	0.17		4.30	[3.58; 5.15]	12.0%
Vaes et al., 2018a	26	10	9.87	1.47	24	20	3.60	0.73		2.74	[2.48; 3.03]	12.7%
Vaes et al., 2018b	12	5	10.44	2.79	11	20	3.58	0.71		2.92	[2.41; 3.53]	5.3%
Vaes et al., 2018b	14	10	8.87	1.40	11	20	3.58	0.71		2.48	[2.15; 2.86]	5.8%
Vaes et al., 2018b	14	15	7.33	0.94	11	20	3.58	0.71		2.05	[1.79; 2.34]	5.9%
Random effects model	**215**				**199**					**2.36**	**[1.82; 3.05]**	**100.0%**
Prediction interval											**[1.09; 5.08]**	

Heterogeneity: I^2 = 98%, τ^2 = 0.105, p < 0.01



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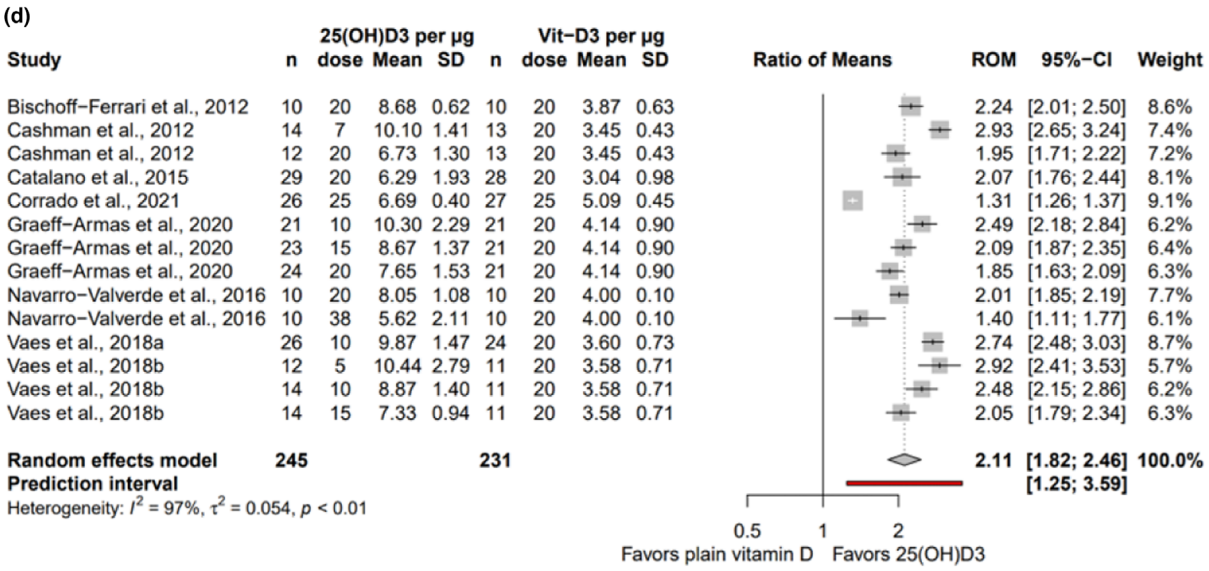

Figure 3: Relative bioavailability of calcidiol monohydrate [25(OH)D_3] compared to vitamin D_3: ratio of means achieved serum 25(OH)D concentration per µg/day of vitamin D administered

Meta-analysis was performed among: (a) RCTs using the same doses of calcidiol and vitamin D_3; (b) all RCTs and intervention arms available; (c) excluding 3 RCTs at high RoB (tier 3); (d) excluding studies using doses ~ 60 µg/day vitamin D_3 as control. 'Mean' and 'SD' refer to study means and standard deviations for the achieved S-25(OH)D concentrations per µg/day of vitamin D, respectively. **Abbreviations**: CI, confidence interval; µg: micrograms; RCT, randomised controlled trial; ROM, ratio of means; SD, standard deviation; S-25(OH)D, serum 25-hydroxyvitamin D; Vit-D_3, vitamin D_3; 25(OH)D_3, 25-hydroxyvitamin D_3, i.e., calcidiol.

Since the dose–response curve between the intake of vitamin D_3 and serum 25(OH)D concentration tends to level-off gradually at high intakes, a sensitivity analysis was conducted excluding the two RCTs that used ~ 60 µg/day vitamin D_3 as the reference dose (Figure 3d). The mean (95% CI) relative bioavailability of calcidiol compared to native vitamin D_3 was 2.11 (1.82, 2.46), with high heterogeneity (I^2 = 97.7%, 95% CI = 96.6–98.0%). This estimate is similar to that obtained when only RCTs using calcidiol and vitamin D_3 at 20 µg/day were considered (ROM, 95% CI: 2.02, 1.85–2.21).

Dose response relationship

A meta-regression analysis was performed to explore the dose–response relationship between the achieved serum 25(OH)D concentrations per µg/day of vitamin D administered and the dose of the two forms of vitamin D (calcidiol vs. vitamin D_3). Doses of calcidiol ranged from 5 to 38 µg/day and doses of vitamin D_3 from 20 to 62 µg/day. The predictor variables were the dose of vitamin D (µg/day), the form of vitamin D (calcidiol vs. vitamin D_3) and their interactive effect. The mean baseline serum 25(OH)D concentrations (nmol/L) was a covariate. Studies and arms were included as nested random factors. All 26 arms from the 10 eligible RCTs were included in the model as coming from an observational setting. Therefore, the control for confounding factors normally achieved with RCTs is not necessarily attained here. For example, the different range of doses for calcidiol and vitamin D_3 is a potential confounder only partially controlled for by standardising the effect (achieved serum 25(OH)D concentrations) by dose. Details on the statistical analysis can be found in the statistical report (see Appendix F of the opinion and Annex F of the external technical report).

None of the model parameters provides evidence for an effect of the dose differential for calcidiol versus vitamin D_3, baseline serum 25(OH)D concentration or the type of vitamin D independent of dose. Using non-linear relationships and restricting the predictor to the dose and/or the type of vitamin D do not change the conclusions.

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3.3.4.3. Effect of calcidiol monohydrate versus vitamin D_3 on serum PTH concentrations

Seven of the twelve eligible RCTs reported serum PTH concentrations in a sufficient detail and were included in the brief evidence synthesis below. One of these RCTs (Ruggiero et al., 2019) could not be included in evidence synthesis for serum 25(OH)D concentrations (see Section 3.3.4.1) and one RCT reported on changes from baseline, rather than on achieved serum PTH concentrations (Vaes et al., 2018a). Two RCTs reporting on serum PTH were not included in the evidence synthesis for this endpoint because one did not report units of measurement (Corrado et al., 2021) and the other was a cross-over with no washout (Jakobsen et al., 2017).

As expected, achieved serum PTH concentrations were generally lower with calcidiol than with vitamin D_3 (Figure 4a), except when calcidiol was administered at much lower doses (i.e., 5 and 7 μg/day) than vitamin D_3 (~ 20 μg/day). This was also the case in one RCT using similar doses of both vitamin D forms (Ruggiero et al., 2019). However, this could be explained by the higher serum PTH concentrations observed in the calcidiol group at baseline (see Appendix C.1).

As for serum 25(OH)D, the mean difference in achieved serum PTH concentrations per μg/day between calcidiol and vitamin D_3 (reference; doses ~ 20 μg/day in all studies) was calculated for all calcidiol arms (Figure 4b). Achieved serum PTH concentrations per μg/day of vitamin D were comparable for calcidiol and vitamin D_3 when both were given at similar doses (~ 20 μg/day), suggesting a similar effect in suppressing PTH synthesis/secretion. At lower doses of calcidiol, however, achieved serum PTH concentrations per μg/day of vitamin D were higher for calcidiol than for vitamin D_3, and decreased with increasing calcidiol dose in a dose–response manner in the four RCTs with multiple calcidiol doses (Cashman et al., 2012; Navarro-Valverde et al., 2016; Vaes et al., 2018b; Graeff-Armas et al., 2020).

Similar results were obtained when the ROM was calculated (Figure 4c). The ratio of means of achieved serum PTH concentrations per μg/day of calcidiol versus vitamin D_3 was close to 1 (0.83 to 1.10) when both were given at 20 μg/day but increased with decreasing doses of calcidiol (from 4.34 to 1.11 for doses 5 to 15 μg/day) in a dose–response manner. This suggests that calcidiol was as efficient as vitamin D_3 in suppressing PTH only when given at similar doses, the effect decreasing at lower doses of calcidiol.

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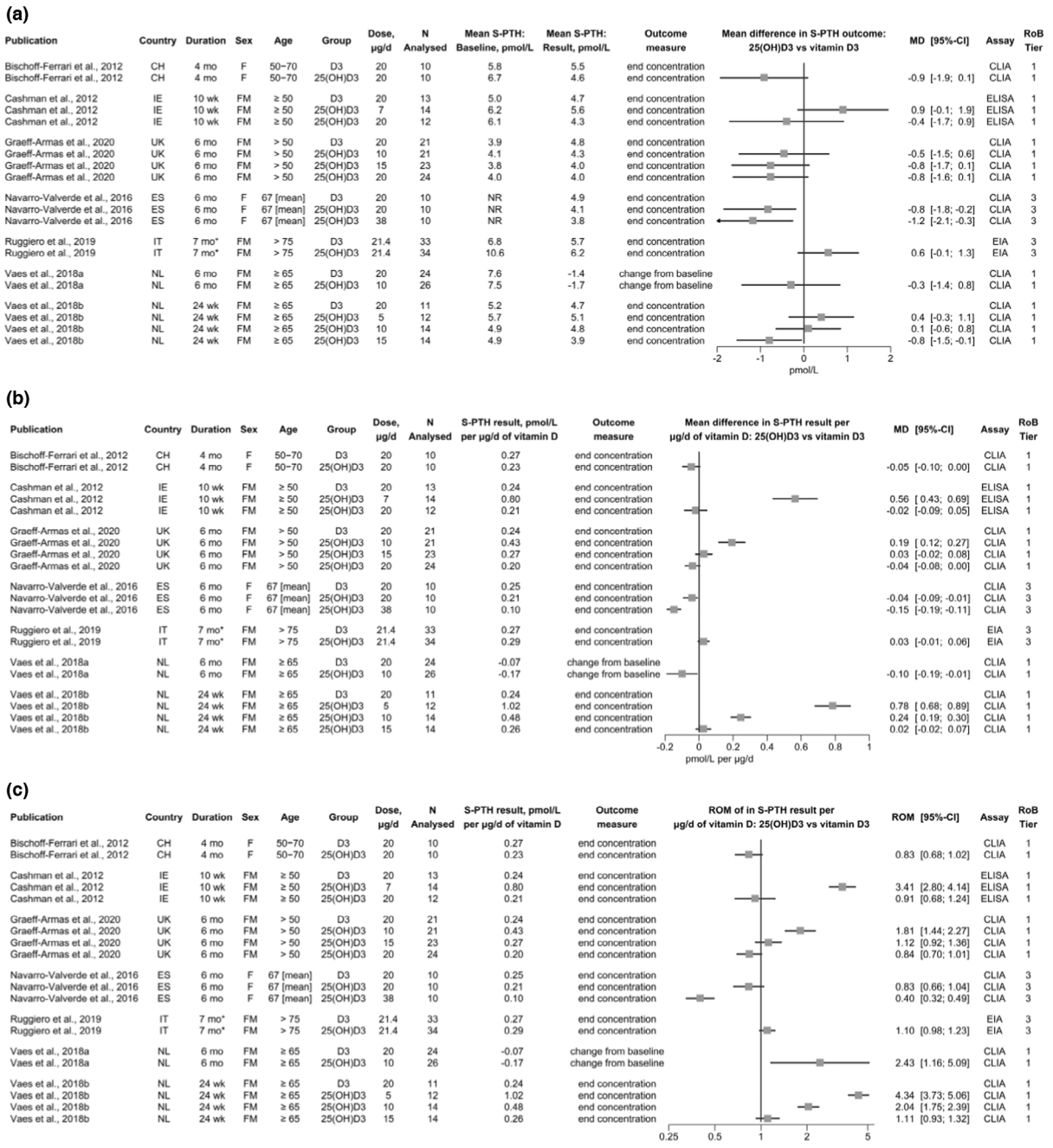

Figure 4: The effect of calcidiol monohydrate on serum PTH concentration compared to vitamin D_3: (a) achieved serum PTH concentration; b) achieved PTH concentration per µg/day of vitamin D; (c) ratio of means (ROM) of achieved PTH concentration per µg/day of vitamin D

Duration as reported by the authors. For age, recruitment target range is presented unless otherwise indicated. Outcome measure: end concentration refers to S-PTH concentration at the end of the treatment; change from baseline concentration refers to difference between end concentration and baseline concentration [end concentration minus baseline concentration]. Mean difference = mean S-PTH concentrations achieved with calcidiol minus those achieved with vitamin D_3. **Abbreviations:** CI, confidence interval; CH, Switzerland; CLIA, chemiluminescence immune assay; D_3, vitamin D_3; EIA, enzyme immunoassay; ES, Spain; F, females; IE, Ireland; IT, Italy; M, males; MD, mean difference; NL, the Netherlands; RoB, risk of bias; S-PTH, serum parathyroid hormone; UK, United Kingdom; 25(OH)D_3, 25-hydroxyvitamin D_3, i.e., calcidiol.

*From hospital admission to 7 months after discharge.

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As discussed in Section 3.3.4.2, calcidiol raised serum 25(OH)D about twice as much than vitamin D_3 when given at similar doses of 20 μg/day. This relative effect on serum 25(OH)D increased with decreasing doses of calcidiol in a dose–response manner, suggesting the relative higher efficacy of calcidiol versus vitamin D_3 in raising serum 25(OH)D, particularly at lower doses. However, the Panel notes that this is not reflected in a similar efficacy in reducing serum PTH concentrations.

3.3.4.4. Derivation of a conversion factor for calcidiol monohydrate

The Panel notes that relative bioavailability of calcidiol versus native vitamin D_3 in equimolar amounts has been assessed only at doses of 20 and 25 μg/day (Figure 3a). At 20 μg/day, the mean relative bioavailability of calcidiol (n = 5 RCTs) is 2.02 (95% CI: 1.85, 2.21) times that of vitamin D_3 and drops to 1.31 (95% CI: 1.26, 137) times at 25 μg/day (only 1 RCT available).

The Panel also notes that the mean relative bioavailability of calcidiol compared to vitamin D_3 obtained in the meta-analysis including all RCTs available and all calcidiol arms (Figure 3b) was 2.4 (95% CI: 1.89, 3.06), dropping to 2.11 (95% CI:1.82, 2.46) when the two RCTs that used ~60 μg/day vitamin D_3 as the reference dose were excluded in sensitivity analyses (Figure 3d).

Taking into account that the use of calcidiol in food supplements has been considered safe at intake levels up to 10 μg/day (EFSA NDA Panel, 2021a), and that the relative bioavailability of calcidiol versus vitamin D_3 consistently increases with decreasing doses of calcidiol in the four RCTs using multiple calcidiol doses (Figure 2c), the Panel considers that a CF of 2.5 better reflects the relative bioavailability of calcidiol as proposed for use in food supplements.

Therefore, the Panel proposes a conversion factor for calcidiol monohydrate into vitamin D_3 of 2.5 for labelling purposes.

The specific conversion factor for calcidiol monohydrate accounts for its higher efficacy in increasing serum 25(OH)D concentrations per unit dose administered as compared to cholecalciferol (vitamin D_3) for doses up to 10 μg/day.

From a scientific point of view, the Panel considers that the biological value of substances with vitamin D activity could be expressed as vitamin D equivalent (VDE), so that 1 μg VDE = 1 μg cholecalciferol (vitamin D_3) = 1 μg ergocalciferol (vitamin D_2) = 0.4 μg calcidiol monohydrate = 40 IU. This applies to calcidiol monohydrate at doses up to 10 μg/day.

3.3.4.5. Uncertainty analysis

The derivation of the CF is based on 10 RCTs that include healthy male and female adults and populations with low and adequate vitamin D status, mostly 50 years of age and older. Since intestinal absorption of vitamin D_3 does not appear to be significantly affected by age (see Section 3.2.2), the Panel considers that the CF for calcidiol derived from these studies could apply to all population groups that are the target population for the use of calcidiol in food supplements (11 years of age and older).

These RCTs were mostly at low RoB (n = 6 in tier 1; n = 1 in tier 2; n = 3 in tier 3), critical domains being exposure characterisation, outcome assessment, allocation concealment and blinding. Whereas 4 RCTs used ELISA, CLIA or an unknown method to assess serum 25(OH)D concentrations and were rated as being at possible high RoB for the outcome assessment, this methodological aspect is expected to have a low impact on the CF derived from individual RCTs and meta-analysis thereof, as serum 25(OH)D was measured by the same method in both the vitamin D_3 and calcidiol arms. Indeed, sensitivity analysis omitting RCTs at high RoB did not substantially modify the results (see Section 3.3.4.2, Figure 3c). However, it may have had an impact on the dose–response meta-analysis, where all the intervention arms were analysed as independent observations. The risk of publication bias was difficult to assess (see Appendix F).

The main uncertainties associated with the proposed CF for calcidiol monohydrate relate to data gaps in the BoE, mostly in relation to the reference dose of vitamin D_3 used in the studies, and the bioequivalence of calcidiol versus vitamin D_3.

Whereas the effect of calcidiol on serum 25(OH)D concentrations (the selected marker of vitamin D status) was assessed over a wide range of intakes in the available RCTs (5 to 38 μg/day), vitamin D_3 was used as reference only at doses of 20–25 μg/day or ~ 60 μg/day. At intakes of calcidiol of 20 μg/day, the dose of vitamin D_3 used as comparator (20 μg/day vs. ~60 μg/day) had a big impact on the relative bioavailability per μg/day of vitamin D administered (~ 2 vs. ~ 4.5). The Panel notes the lack of eligible studies comparing equimolar doses of calcidiol versus vitamin D_3 at <20 μg/day, which could provide a better estimate of the CF for calcidiol monohydrate over that range of intake.

Serum PTH concentrations were reported in seven RCTs (see Section 3.3.4.3). The consistent finding that calcidiol was more effective in increasing serum 25(OH)D concentrations than vitamin D_3

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but not in concomitantly suppressing serum PTH concentrations reflects the need to elucidate further the biological activity of the two forms of the vitamin.

The Panel notes that higher CFs for calcidiol monohydrate into vitamin D_3 have been previously proposed (e.g., Bouillon and Quesada Gomez (2023), who propose a CF of 3). The Panel also notes that the dose, frequency and duration of supplementation with both calcidiol monohydrate and vitamin D_3 are likely to have an impact on achieved serum 25(OH)D concentrations, and thus on the relative bioavailability of calcidiol monohydrate versus the reference (vitamin D_3). Hence, the criteria for study selection in this opinion regarding the frequency of supplementation (daily or weekly doses) and the minimum duration of the intervention (6 weeks) lead to the exclusion of RCTs that have been considered for the purpose of deriving a CF for calcidiol monohydrate in other assessments and may explain the different conclusions reached.

3.4. Intake assessment

This section provides harmonised intake estimates of vitamin D naturally present in foods (i.e., from the background diet) across European countries calculated using the EFSA Comprehensive food consumption and the EFSA food composition databases, following extensive data cleaning to exclude fortified foods (Section 2.2.2). Data available to EFSA in such databases were insufficient to provide harmonised intake estimates of vitamin D from fortified food and/or food supplements. Published data from national food consumption surveys is presented instead.

3.4.1. Dietary sources of vitamin D

Natural sources

Vitamin D is naturally found in food as ergocalciferol (D_2) and cholecalciferol (D_3). Animal-derived foods such as fatty fish, fish offal, fish oil, and egg yolks are particularly rich in vitamin D_3, whereas vitamin D_2 is mostly found in sources of plant origin, including some higher fungi such as mushrooms. Vitamin D_2 is produced in fungi by UV-B exposure of provitamin D_2 and the content depends on the amount of UV-B light and the time of exposure (Kristensen et al., 2012; Tangpricha, 2012). Calcidiol is naturally found in some products of animal origin, such as meat and meat products (particularly offal), dairy (particularly milk and butter), fish, and eggs (Jakobsen and Saxholt, 2009; Cashman, 2012; Benedik, 2022).

The content of total vitamin D naturally present in specific foods of animal and vegetable origin within these food categories can be highly variable and is presented in Annex B.

Fortified foods

In the EU, authorised forms of vitamin D for addition to foods are ergocalciferol and cholecalciferol.[13] EU regulations set minimum and maximum content of vitamin D in infant and follow-on formulae, and in processed cereal-based foods for infants and children.[14]

In the Mintel GNPD (from November 2017 to November 2022), a total of 6,169 packaged food products available in 24 EU Member States and Norway were identified as containing added vitamin D in the ingredients list. Most products belong to the Mintel categories 'dairy' (42%, includes dairy alternatives, median 1.13 μg/serving), 'nutritional drinks and other beverages' (12%, median 1.8 μg/serving) and 'breakfast cereals' (10%, median 0.9 μg/serving). The highest vitamin D content declared in the label was found in three meal replacement drinks under the category 'nutritional drinks and other beverages' (20–25 μg/serving), two breakfast cereals (13.2 and 15.3 μg/serving), nine cereal bars under the category 'snacks' intended for breastfeeding mothers (10 μg/serving), and two fortified juice drinks (11.9 and 10 μg/serving).

[13] 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.

[14] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive1999/21/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, 6.12.2006, p. 16.

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Food supplements

In the EU, authorised forms of vitamin D for use in food supplements are ergocalciferol and cholecalciferol.[15] A search in the Mintel GNPD (from November 2017 to November 2022) yielded a total of 2,150 products available in 'vitamins and dietary supplements' category across 24 EU Member States and Norway. The median dose declared on labels was 10 μg/serving. About 67% of supplements contained up to 15 μg vitamin D per serving, and 0.1% had doses > 100 μg per serving, with a maximum of 140 μg per serving (Figure 5).

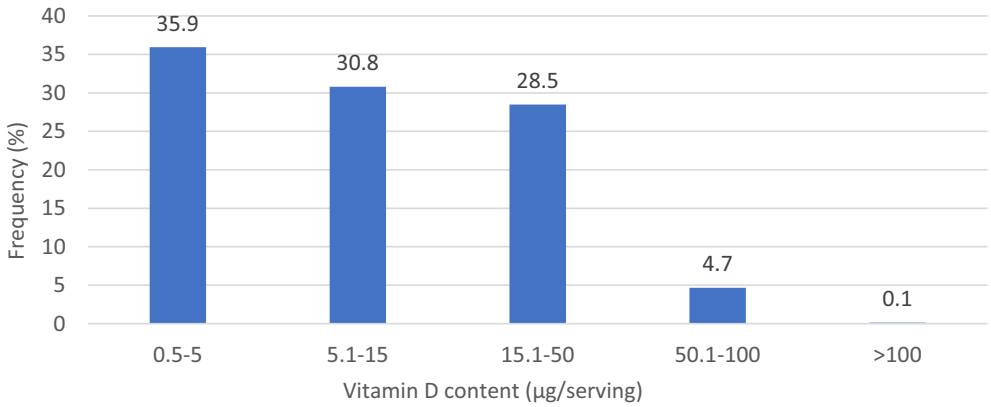

Figure 5: Distribution of vitamin D content in food supplements as displayed on labels in EU Member States and Norway (μg/serving)

Source: Mintel GNPD. Search for vitamin D-containing supplements available in the EU market in the last 5 years (from November 2017 to November 2022). A total of 2,150 products available in 24 EU Member States and Norway were identified, of which 2,098 contained complete data on μg/serving.

3.4.2. EFSA's intake assessment on background intake

Dietary intakes of vitamin D in μg/day from natural food sources (background intake) were calculated linking food consumption data at individual level in the EFSA Comprehensive Database to food composition data and by using the observed individual means method.

The intake estimates are presented below by age group, sex, and country (Figures 6, 7 and 8). A summary overview, providing the range of means and 95th percentiles (P95) across EU surveys is given in Table 5.

[15] 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.

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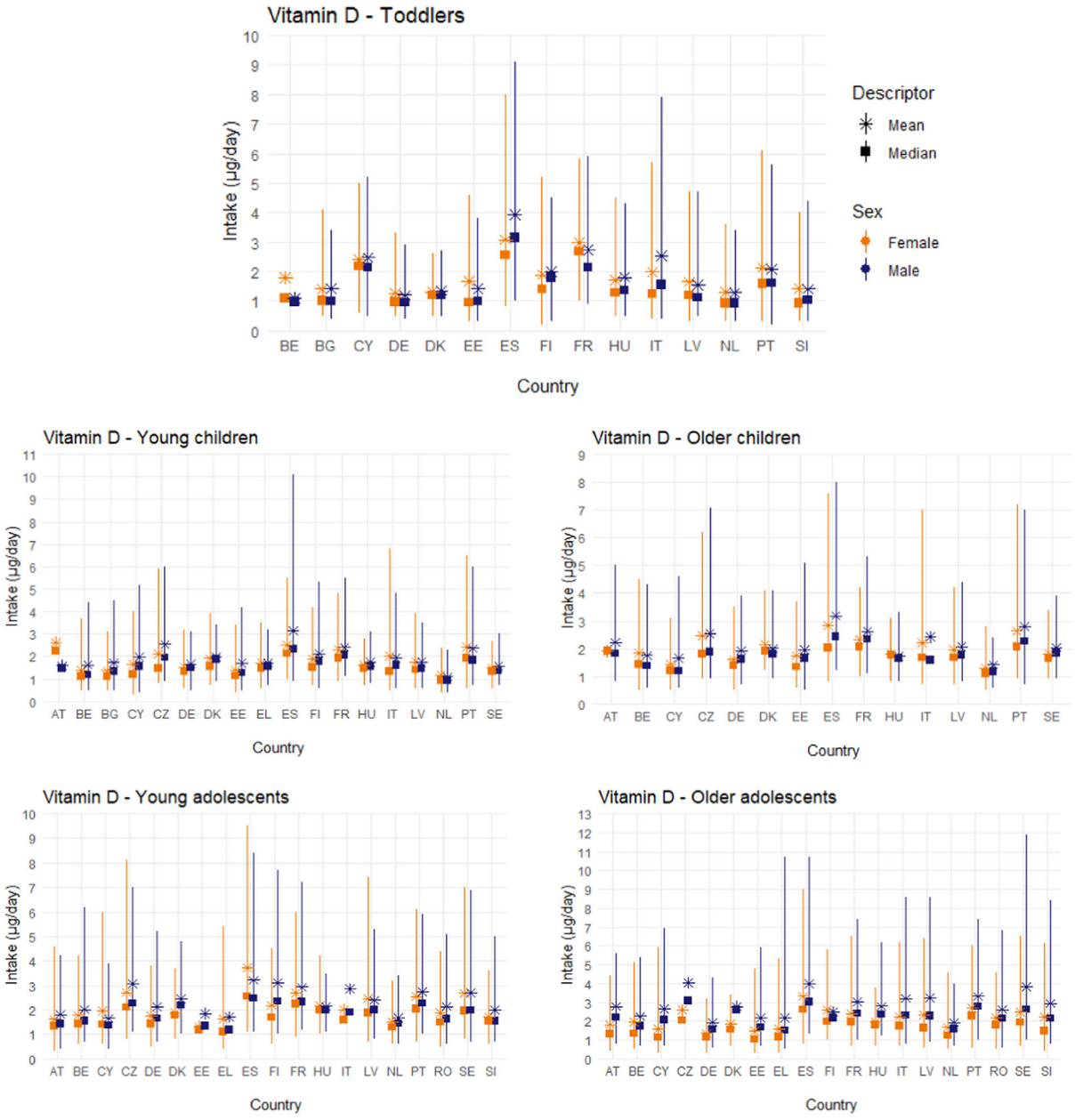

Figure 6: Mean, median, 5th and 95th percentiles of background vitamin D intakes in toddlers (≥ 1 year to < 3 years old), young children (≥ 3 years to < 7 years old), older children (≥ 7 years to < 10 years old), intakes in young adolescents (≥ 10 to < 14 years) and older adolescents (≥ 14 to < 18 years), by sex and country

Estimates for females in orange and for males in blue. Squares correspond to medians and stars to means. Lines represent the range between the 5th and 95th percentiles. Estimated intakes from 5th and 95th percentiles are not presented when sample size is below 60 participants. **Abbreviations**: AT, Austria; BE, Belgium; BG, Bulgaria; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; ES, Spain; FI, Finland; FR, France; HU, Hungary; IT, Italy; LV, Latvia; NL, the Netherlands; PT, Portugal; RO, Romania; SE, Sweden; SI, Slovenia.

*Country for which more than one survey was available; estimates presented in the plot are those of the most recent survey; when surveys covered the same period those, with the highest number of participants are displayed.

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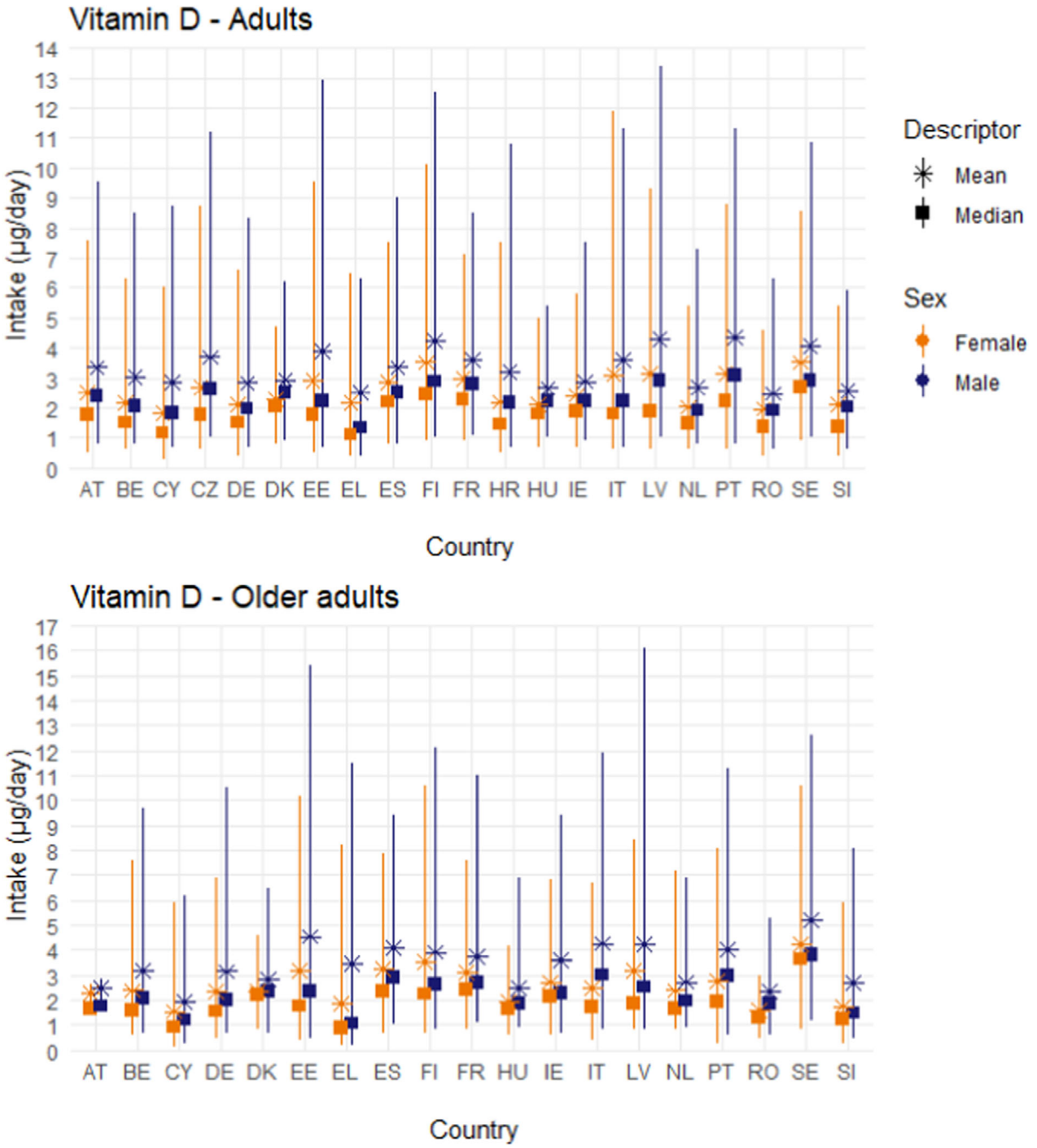

Figure 7: Mean, median, 5th and 95th percentiles of background vitamin D intakes in adults (≥ 18 years to < 65 years old) and older adults (≥ 65 years), by sex and country.

Estimates for females in orange and for males in blue. Squares correspond to medians and stars to means. Lines represent the range between the 5th and 95th percentiles. Estimated intakes from 5th and 95th percentiles are not presented when sample size is below 60 participants. **Abbreviations**: AT, Austria; BE, Belgium; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; EL, Greece; ES, Spain; FI, Finland; FR, France; HR, Croatia; HU, Hungary; IE, Ireland; IT, Italy; LV, Latvia; NL, the Netherlands; PT, Portugal; RO, Romania; SE, Sweden; SI, Slovenia.

*Country for which more than one survey was available; estimates presented in the plot are those of the most recent survey; when surveys covered the same period those with the highest number of participants are displayed.

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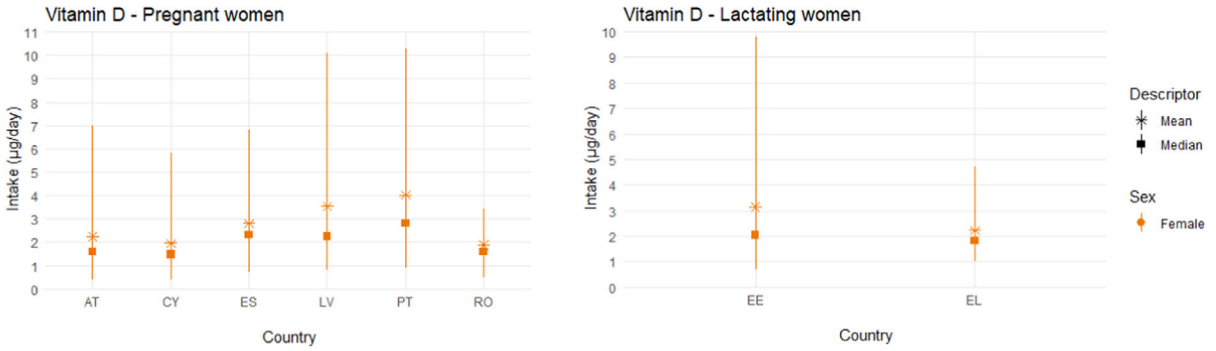

Figure 8: Mean, median, 5th and 95th percentiles of background vitamin D intakes in pregnant and lactating women, by country

Squares correspond to medians and stars to means. Lines represent the range between the 5th and 95th percentiles. Estimated intakes from 5th and 95th percentiles are not presented when sample size is below 60 participants. **Abbreviations:** EE, Estonia; EL, Greece.

Table 5: Daily intake of vitamin D from food sources (supplements and fortified foods excluded) across European dietary surveys, by population group (µg/day)

Population group, age range	N of surveys	Males				Females			
		Mean		P95[(a)]		Mean		P95[(a)]	
		Min.[(b)]	Max.[(b)]	Min.[(b)]	Max.[(b)]	Min.[(b)]	Max.[(b)]	Min.[(b)]	Max.[(b)]
Toddlers, ≥ 1 to < 3 y	15	1.10	3.93	2.75	9.12	1.28	3.09	2.56	7.97
Young children, ≥ 3 to < 7 y	20	1.10	3.15	2.25	10.1	1.15	2.63	2.12	6.76
Older children, ≥ 7 to < 10 y	15	1.42	3.18	2.39	7.97	1.30	2.83	2.81	7.58
Young adolescents, ≥ 10 to < 14 y	20	1.66	3.22	3.39	8.37	1.32	3.72	3.19	9.46
Older adolescents, ≥ 14 to < 18 y	19	1.93	4.05	4.02	11.9	1.35	3.33	3.23	9.00
Adults, ≥ 18 to < 65 y	22	2.48	4.34	5.43	13.4	1.84	3.53	4.57	11.9
Older adults, ≥ 65	23	1.60	5.21	5.26	16.1	1.37	4.25	3.05	10.6
Pregnant women	6					1.90	4.03	3.38	10.3
Lactating women	2					2.23	3.15	4.72	9.83
Vegetarians[(c)]	1	1.16	1.16	3.24	3.24	0.76	0.76	1.96	1.96

mo: months; n: number; P, percentile; y: years.
(a): The 95th percentile estimates obtained from dietary surveys and population groups with fewer than 60 subjects may not be statistically robust (EFSA, 2011a) and consequently are not considered in this table.
(b): Minimum and maximum mean and 95th percentile estimates across European surveys, for each population group.
(c): Age range (12–70 years).

The top food group contributing to total vitamin D intake was 'fish, seafood and their products" for almost all age and population groups across countries. Foods consumed under this group include mainly fatty fish and processed products such as canned and smoked fish; the exceptions were toddlers, for whom the food group which contributed the most was 'food products for young population' (mainly infant and follow-on formulae) and for vegetarians, for whom the highest contributor was 'eggs and egg products'. Other food groups which mainly contributed to the total vitamin D intake across population groups and countries were 'eggs and egg products' (mainly whole hen egg), 'milk and dairy products' (mainly cow milk, dairy desserts and cheese), and 'meat and meat products' (pig, bovine and turkey fresh meat, processed products such as sausages and other cured or seasoned meats). The contribution of 'milk and dairy products' tended to decrease from younger to older age groups while the contribution of 'meat and meat products' increased with age in most

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countries. Additionally, for children and adolescents, 'grains and grain-based products' were high contributors to the total intake in some surveys due to the consumption of bakery wares such as cakes, pies and pastries. Differences in main contributors to total vitamin D intake between genders were in most cases minor. Other food groups adding up to the total intake of vitamin D are presented in Annex B.

According to the intake assessment protocol (EFSA, 2022), EFSA's estimates have been compared with published national vitamin D background intake estimates from the same surveys with the same (or similar) window of data collection and population groups, when available (Section 2.2.1). In most cases, mean and P95 intakes for vitamin D calculated by EFSA were in the same range with those from background diet only reported in published reports from national surveys (Section 3.4.3). However, few national surveys assessed vitamin D intake from background diet only (3 countries).

EFSA's intake estimates were generally lower than national intake estimates including foods fortified with vitamin D (18 countries), indicating that fortified foods have been successfully excluded from the food composition database to estimate background intakes.

3.4.2.1. Sources of uncertainty

Sources of uncertainty and their potential impact on the intake estimates, where possible, are identified and further discussed in Annex C.

Specific to this intake assessment, uncertainties arise from the incomplete information and lack of harmonisation in EFSA's FCDB on: the vitamin D form/component reported ('vitamin D' instead of 'total vitamin D', 'cholecalciferol', 'ergocalciferol' or '25-hydroxycholecalciferol'); the method of analysis or calculation of vitamin D (e.g. 'Analytical or calculation method not known'); and/or on the lack of detail of the original food name and the food name descriptors (e.g. 'freshwater fish').

For this opinion, food composition data from 12 European countries were pooled, which may cover up specific country differences in the vitamin D concentration of different foods. However, this approach allowed for more food products to be considered per food category, leading to a more robust database which considers product variability, assuming a global food market.

Additionally, as the scope of intake assessment was to consider natural sources of vitamin D only, a data cleaning strategy was applied to exclude fortified foods from the composition database. Since fortification was not always clearly reported, assumptions had to be made to exclude suspected fortified foods (e.g. by identifying outlying values). These assumptions could result in both an overestimation or underestimation of the background intake of vitamin D. However, the impact of this uncertainty is expected to be small as the levels of vitamin D reported in the final food composition database used in this assessment were similar to those reported in other national food composition databases. Moreover, while EFSA's intake assessment estimates are in line with national estimates which reported on the intake from the background diet only, they are generally lower than those national estimates which included both natural sources of vitamin D and fortified foods.

3.4.3. Information on fortified foods and food supplements

Data on vitamin D intake from fortified foods and food supplements were collected from nationally representative food consumption surveys by contacting 64 competent authorities in 27 European countries (Section 2.2.1). For surveys that did not clearly indicate whether fortified foods were included in the estimates, it was assumed that they were included. Survey characteristics, mean and P95 intake estimates are presented in Annex D. Key information is summarised in the following paragraphs.

3.4.3.1. Intake from fortified foods

Different mandatory and voluntary fortification practices are in place in EU countries. In Sweden it is mandatory to add vitamin D to margarine and fat blends, drinking milk and fermented milk products with a fat content not exceeding 3% of fat by weight); in Belgium, to margarine, low-fat margarine and fats for baking; in Finland, to skimmed homogenised milk and organic milk; and in Poland, to margarine with normal and reduced fat content, mixtures of butter and oil. In other countries, vitamin D is voluntarily added mainly to fats and oils and milk and milk products. Details on the amount of vitamin D added to foods in each EU country can be found in Appendix G.

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Intake estimates from national food consumption surveys

Reports from national consumption surveys providing estimates of vitamin D intake from food, including fortified foods but excluding food supplements, are available from 26 surveys in 19 countries: Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Latvia, Lithuania, Netherlands, Norway, Serbia, Slovenia, Spain, and Sweden.

Estimated intakes for females were generally lower than for males in all studies and age groups (see Annex D). The highest P95 values in males from foods and fortified foods were reported in France for toddlers (11.7 μg/day), in Belgium for all children (7.8 μg/day) and in Sweden for all adolescents (13.1 μg/day). For adults, highest P95 values were reported in Denmark (18.6 μg/day), and for older adults in Sweden (19.5 μg/day).

Contribution of fortified foods to total vitamin D intake

Among the national food consumption surveys available, only two distinguish between vitamin D intake from natural sources and vitamin D intake from fortified foods.

In the Netherlands, fats are encouraged to be fortified with vitamin D through voluntary fortification. Specific margarines and other spreadable fats intended for persons > 60 years may contain at least 0.2 μg and a maximum of 0.25 μg vitamin D/g product. In the national food consumption survey, 84% of the Dutch population reported to use vitamin D-fortified fats. Among users, the median contribution of fortified fats to total vitamin D intake was 44%, and the P95 reached about 86% (de Jong et al., 2022).

In Belgium, margarines and spreadable fats are mandatorily fortified with vitamin D at levels ranging from 6 to 7.5 μg/100 g. Mandatorily-fortified foods contributed between 3.2% and 13.9% to total vitamin D intake. Voluntary fortified foods contributed between 6.6% and 14.8% to total vitamin D intake in children and adolescents, and between 2.7% and 5.7% in adults (Moyersoen et al., 2017).

3.4.3.2. Intake from food supplements

Nutritional guidelines or recommendations at national level in the EU differ across countries. Details on the amounts recommended for specific age and population groups can be found in Appendix G.

Information on vitamin D intake from all sources, including food supplements, is available for 16 dietary surveys conducted in 10 countries: Belgium, Denmark, Finland, Germany, Ireland, the Netherlands, Norway, Poland, Sweden and Slovenia. Study characteristics and intake estimates are presented in Annex D.

Data collected on the use of vitamin D supplements in EU surveys are briefly summarised below.

Intake of vitamin D in supplement users

Toddlers, other children and adolescents

Data on the use of vitamin D supplements in toddlers, other children and adolescents were available from eight national dietary surveys conducted in Denmark, Germany, Ireland, the Netherlands, Norway and Slovenia. A summary of the data collected are provided in Table 6.

In children and adolescents of different age groups, the contribution of food supplements to total vitamin D intake in users ranged from 32% in Ireland (13–18 years old) to 80% in Denmark (4–10 years old). Intakes from food supplements in high consumers (P95) were up to 25 μg/day in Ireland in adolescents aged 13–18 years old.

Absolute intakes from all sources in supplement users were reported in Denmark and Sweden only. Median intakes from all sources were 9.2 μg/day (P95: 30 μg/day) in Denmark in female adolescents aged 11–17 years, while mean intakes in Sweden were 27 μg/day (P95 not reported) in adolescents aged 12–16 years of mixed sex (Annex D).

Adults

Nine national dietary surveys conducted in Belgium, Denmark, Finland, Germany, Ireland, Norway, Poland and Sweden reported on the use of vitamin D supplements in adults (Table 7). The lowest and highest contribution of food supplements to total vitamin D intake was observed in Ireland (28%) and Denmark (80% in females), respectively. Intakes from food supplements were up to 50 μg/day (median) in Poland among adult females (P95 not reported; min-max: 5–100 μg/day) (Anex D).

Absolute intakes from all sources in supplement users were reported in Denmark, Germany, Finland and Sweden only, with intakes ranging between 5.6 μg/day (median) in Germany and 36 μg/day (mean) in Finland (highest reported P95 in Danish females: 54 μg/day) (Annex D).

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Table 6: Percent vitamin D supplement users in European surveys and vitamin D intake from food supplements among users (toddlers, children and adolescents)

Country Survey name (N subjects) Reference	Dietary method, (N of days)	Sex	Age range	% vitamin D supplement users in total survey sample/ among supplements users	Vitamin D intake from supplements, P95 (µg/day)	Contribution of supplements to vitamin D intake, mean (%)
Denmark DANSDA 2011–2013 (n = 3936) (Hindborg, 2015, Unpublished)	Face-to-face interview	m + f m f	4–10 y 11–17 y 11–17 y	62/NR 50/NR 48/NR	NR	80 75 79
Germany EsKiMo 2015–2017 (n = 2644) (Perlitz et al., 2019; Mensink et al., 2021)	Short questionnaire + weighing logs	m + f	12–17 y	6.7/NR	NR	NR
Ireland NPNS 2011–2012 (n = 500) NCFS II 2017–2018 (n = 600) NTFS II 2019–2020 (n = 428) (Kehoe et al., 2022)	Weighted food diary (4d)	m + f	1–4 y 5–12 y 13–18 y	17/79 19/84 7/52	10 14.8 25	46 45 32
The Netherlands DNFCS 2012–2016 (n = 4313) (van Rossum et al., 2020)	Questionnaire (online/paper)	m	1–3 y 3–10 y 10–14 y 14–18 y	77/89 33/58 15/38 10/29	13.2 14.4 7.3 4.8	NA NA NA
		f	1–3 y 3–10 y 10–14 y 14–18 y	76/19 33/38 21/37 12/22	11.8 18.2 10.3 15.6	NA NA NA NA
Norway Småbarnskost 2019 (n = 1,413) Ungkost 3, 2015 (n = 687) (VKM, 2022)	FFQ + food diary +24-h dietary interviews	m + f	2 y 9–13 y	16/NR NR/NR	Mean 5.4 3.1	55 NR
Slovenia SI.Menu 2017–2018 (n = 1248) (Hribar et al., 2021)	FPQ	m + f	10–17 y	Multivitamins/ Vitamin D only 72/17	NR	NR

Abbreviations: d, day; DANSDA, The Danish National Survey of Diet and Physical Activity; DNFCS, Dutch National Food Consumption Survey; EsKiMo, Eating study as a KiGGS Module; f, females; FFQ; food frequency questionnaire FPQ, food propensity questionnaire; N, m, males; number; NA, cannot be calculated; NCFS, National Children's Food Survey; NPNS, National Pre-School Nutrition Survey; NR, not reported in the publication, NTFS, National Teen's Food Consumption Survey; SD, standard deviation; VKM, Vitenskapskomiteen for mat og miljø; y, year.

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Table 7: Percent vitamin D supplement users in EU surveys and vitamin D intake from food supplements among users (adults and older adults)

Country Survey name (N subjects) Reference	Dietary method (N of days)	Sex	Age range	% vitamin D supplement users in total survey sample/ among supplements users	Vitamin D intake from supplements, P95 (µg/day)	Contribution of supplements to total vitamin D intake, mean (%)
Denmark DANSDA 2011–2013 (n = 3936) (Hindborg, 2015. Unpublished)	Face-to-face interview	m f m f	18–50 y 18–50 y 51–75 y 51–75 y	47/NR 57/NR 44/NR 68/NR	NR	74 80 73 80
Finland FINDIET 2017 (n = 1,655) (Valsta et al., 2018)	FPQ	m f	18–74 y	40/NR 57/NR	Mean (µg/day) 23 26	64 72
Germany NVS II 2005–2007 (n = 13,753) (Heuer et al., 2012)	24-h recall (2d)	m f	15–80 y	4.0/NR 6.5/NR	10 15	Median 45 59
Ireland NANS 2008–2010 (n = 1,500) (Kehoe et al., 2022)	Weighted food diary (4d)	m + f	18–64 y 65–91 y	16/52 27/71	15 25	28 41
The Netherlands DNFCS 2012–2016 (n = 4,313) (van Rossum et al., 2020)	Questionnaire (online/paper)	m	18–65 y 65–80 y	15/41 16/48	17.8 25.3	NA
		f	18–65 y 65–80 y	26/44 36/60	20.9 27.2	NA
Norway Norkost 3 2015 (n = 1,787) (Totland et al., 2012; VKM, 2022)	FFQ + food diary +24-h dietary interviews	m + f	18–70 y	47/(m) 58/(f)	Mean 5 5	NA
Poland National Dietary Survey 2019–2020 (n = 1,831) (Stos et al., 2021)	FPQ	m f	18–65+ y	NR/36 NR/55	Mean ± SD (range) 15.7 ± 20.4 (2.5–60) 33.1 ± 26.4 (5–100)	NA
Slovenia SI.Menu 2017–2018 (n = 1,248) (Hribar et al., 2021)	FPQ	m + f	18–64 y 65–74 y	Multivitamins/ vitamin D only 52/22 11/20	NR	NR
Sweden Riksmaten 2010–2011 (n = 1,797) (SFA, 2015. Unpublished)	Dietary records (x4)	m + f	18–80 y	NR/3	NR	NA

Abbreviations: d, day; DANSDA, The Danish National Survey of Diet and Physical Activity; DNFCS, Dutch National Food Consumption Survey; f, females; FINDIET, The Finnish National Dietary Survey in Adults and Elderly; FFQ; food frequency questionnaire FPQ, food propensity questionnaire; N, number; m, males; NA, cannot be calculated; NANS, National Adult Nutrition Survey; NR, not reported in the publication; NVS II, Nationale Verzehrsstudie II; SD, standard deviation; VKM, Vitenskapskomiteen for mat og miljø; y, year.

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3.4.4. Overall conclusions on intake data

Intakes of vitamin D from the background diet (i.e. excluding food fortification and food supplements) were estimated using harmonised European food composition and consumption data. The Panel notes that the P95 in males is up to 9 μg/day in toddlers (1 to < 3 years), up to 10 μg/day in young children (3 to < 7 years), up to 8 μg/day in older children (7 to < 10 years) and young adolescents (10 to < 14 years), up to 12 μg/day in older adolescents (14 to < 18 years), and up to 13 μg/day in adults (≥ 18 years). In pregnant and lactating women across the surveys included in EFSA's intake assessment, intakes were up to 10 μg/day (Table 5) (Annex B). Vitamin D intakes from the background diet are slightly lower among females, mainly due to the smaller quantities of food consumed.

Mandatory vitamin D food fortification policies are in place in some EU countries (e.g. Sweden, Belgium, Finland and Poland) mostly for dairy food products and blended fats with reduced fat content (mainly margarines). In other countries, certain foods can be fortified with vitamin D on a voluntary basis, with different amounts depending on national policies (Appendix G). Vitamin D can also be found in food supplements. The Mintel GNPD indicates substantial variability in the amount of vitamin D per serving in food supplements in the European market, with most values being between 0.5 and 10 μg. About 0.1% of the products identified contain > 100 μg (maximum 140 μg) per serving as reported in the label. National nutritional guidelines/recommendations for supplementing the diet with vitamin D (source and amounts) also differ across population groups and countries (Appendix G).

Data on the intake of vitamin D from fortified foods and food supplements were obtained from published national surveys in European countries and are scarce. Only two surveys were available assessing the intake of vitamin D from food (excluding supplements). The contribution of (mandatory or voluntarily) fortified foods to vitamin D intake from food was very variable, up to 86% in consumers of fortified fat spreads in the Netherlands. Among national surveys reporting on total vitamin D intake from all sources, including fortified foods and food supplements, the highest P95 in supplement users was 30 μg/day in children, and 54 μg/day in adult females, both in Denmark. In this country, food supplements contributed up to 80% of total vitamin D intake in children aged 4–10 years.

3.5. Hazard identification

Priority adverse health effects, namely persistent hypercalcaemia and hypercalciuria (sQ3a), and musculoskeletal health (sQ4a/b/c) which includes the risk of bone fractures and of falling in adults 55 years of age and older, as well as BMD, BMC and indices of bone strength at all ages, were addressed through a systematic review of the literature. A description of the processes applied for evidence retrieval, study selection and data extraction is provided in Section 2.1.1 of this opinion (see also the technical report [Lamberg-Allardt et al., 2023]).

3.5.1. Persistent hypercalcaemia and hypercalciuria

The relationship between high intakes of vitamin D and development of persistent hypercalcaemia and/or hypercalciuria is well established (IOM, 2011; EFSA NDA Panel, 2012b); hence the assessment focused on the characterisation of the dose–response relationship.

Eligible studies were RCTs investigating the relationship between vitamin D supplementation and persistent hypercalcaemia/hypercalciuria, as defined by the authors, with an intervention period of at least six weeks (i.e. the time estimated to reach plateau serum 25(OH)D concentrations after the start of the intervention). The definition for persistent hypercalcaemia/hypercalciuria was often unclear in the studies identified; therefore, the approach outlined in Table 8 was implemented and as such, a case of persistent hypercalcaemia/hypercalciuria was defined as a participant with elevated calcium concentrations in blood/urine (as defined within each study) that were confirmed through repeated testing, or who experienced recurrent elevated levels during the study period. Transient cases (i.e. which resolved on re-testing or subsequent follow-up visits) were not included in the analysis, while cases that were unclear as to whether they were transient or persistent were included in the evidence synthesis but specifically noted as uncertain and excluded in sensitivity analyses, if applicable.

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Table 8: Definitions for persistent hypercalcaemia and hypercalciuria cases[(a)]

Persistent cases	Transient cases	Unclear cases	Zero (persistent) cases
• Serum/urine Ca was measured *in all participants* at least twice after baseline and either of the following: • it was reported that retesting for elevated Ca concentration/ excretion was performed, i.e., the result was confirmed by retesting within a given timeand • the elevated concentration/excretion persisted (NB: the participants were excluded from the original study if elevated concentration/excretion persisted after retesting) or • the number of participants who developed recurrently elevated Ca concentration/excretion was reported • i.e., Ca concentration/ excretion was elevated at least at two measurement points • this might be despite the fact that retesting showed resolved concentrations/excretion	• Serum/urine Ca was measured *in all participants* at least twice after baseline and either of the following: • it was stated that retesting for elevated Ca concentration/ excretion was performed, i.e., the result was confirmed by retesting within a given timeand • the elevated concentration/ excretion was resolved, and the participant continued in the studyor • it was stated that the detected hypercalcaemia/ hypercalciuria case(s) was (were) transient	• Serum/urine Ca was measured *in all participants* at least twice after baseline and • it was unclear whether elevated Ca concentration/excretion persisted because > publications did not mention retesting for elevated Ca concentration/excretion > publications only stated the number of hypercalcaemia/ hypercalciuria cases that were observed during intervention period, i.e., protocols for dealing with participants with elevated Ca concentration/excretion were not provided	• serum/urine Ca was measured *in all participants* at least twice after baseline and either of the following: • it was stated that no cases of hypercalcaemia/ hypercalciuria were detected during the entire intervention period or • it was stated that Ca concentrations/excretion remained within the reference range in all participants throughout the intervention period or • it was stated that the hypercalcaemia/ hypercalciuria cases were transient (please see the second column on the left for the definition)

Abbreviation: Ca, calcium.
(a): The participants who developed persistent hypercalcaemia/hypercalciuria.

Studies have utilised several methods of measurement to determine hypercalciuria, including 24-h urine, spot urine calcium concentrations, and urine calcium to creatinine ratios. The gold standard is considered to be 24-h urine measurements; however the other measurements are well accepted as reliable indicators for monitoring hypercalciuria.

As detailed in Section 2.1.1.3, data were not extracted for 31 RCTs investigating vitamin D doses $<$ 100 μg/day in adults and $<$ 50 μg/day in children (i.e., below the current UL for vitamin D for the respective population groups). This is because, below these values, cases of hypercalcaemia or hypercalciuria did not occur, were not persistent, and/or could not be related to the vitamin D dose administered (i.e., the treatment group in which it occurred was not specified in the publication, the number of cases was higher at lower doses of vitamin D, and/or persistent cases occurred in patients with primary hyperparathyroidism). Of these, 27 tested supplemental doses of vitamin D $\leq$ 50 μg/day and only four investigated doses between 60 and 75 μg/day (Gallagher et al., 2014b; Nygaard et al., 2014; Jorde et al., 2016; Jorde et al., 2019). The intervention period ranged from 6 weeks (Himmelstein et al., 1990) to 5 years (Jorde et al., 2016). In all RCTs, the population sampled were adults, except for Rajakumar et al. (2015) where participants were children aged 8–14 years. The

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vitamin D form investigated was calcidiol in five studies (Cashman et al., 2012; Minisola et al., 2017; Vaes et al., 2018b; Graeff-Armas et al., 2020; Gonnelli et al., 2021) and either vitamin D_2 or D_3 in the remaining studies. Doses of calcidiol ranged from 5 to 40 μg/day (Minisola et al., 2017). None of these 31 RCTs indicated an increased risk of persistent hypercalcaemia and/or hypercalciuria with vitamin D supplementation at the doses tested. For a more detailed description of these studies see section 3.3.1.4 of the technical report (Lamberg-Allardt et al., 2023). For a list of these references see Appendix E.

A total of 34 RCTs (reported in 37 publications) investigating vitamin D supplementation doses ≥ 100 μg/day in adults and ≥ 50 μg/day in children were eligible for this assessment. Of these, 33 RCTs monitored hypercalcaemia and 14 monitored hypercalciuria, while 13 RCTs assessed both outcomes (i.e., 20 RCTs investigated only hypercalcaemia and one only hypercalciuria). Some of these studies had multiple doses of vitamin D (including doses < 50 and 100 μg/day), and within-study dose–responses are also presented in the assessment below. The evidence tables are available in Appendix C.2. The list of RCTs reported in multiple publications can be found in Appendix D.

Owing to differences in calcium and vitamin D metabolism and in the physiological requirements for calcium across life stages, the evidence was synthesised separately for each population group i.e., children and adolescents, pregnant and lactating women, and adults.

3.5.1.1. Children and adolescents

Six RCTs investigated the effect of vitamin D supplementation on serum calcium concentrations in children and/or adolescents aged 5–18 years, of which only one in children aged 9–13 years also reported on the development of hypercalciuria (Lewis et al., 2013). Three RCTs were conducted in the USA (Belenchia et al., 2013; Lewis et al., 2013; Rajakumar et al., 2020), one in Iran (Asghari et al., 2021), one in Lebanon (Maalouf et al., 2008) and one in Sri Lanka (Samaranayake et al., 2020). Four RCTs included only overweight or obese children (Belenchia et al., 2013; Rajakumar et al., 2020; Samaranayake et al., 2020; Asghari et al., 2021), while BMI status was unclear in two RCTs (Maalouf et al., 2008; Lewis et al., 2013). Five RCTs investigated multiple doses of vitamin D.

The highest dose of vitamin D administered in these studies ranged from 50 μg/day to 179 μg/day, which was vitamin D_3 in five studies and vitamin D_2 in one. None of the RCTs had calcium co-supplementation. The duration of the intervention was between 3 and 12 months. Mean baseline serum 25(OH)D concentrations were ≥ 50 nmol/L only in one RCT and between 25 and < 50 nmol/L in five. In the three RCTs that had defined serum calcium cut-off points for hypercalcaemia, these ranged between 2.55 and 2.7 mmol/L (see evidence table in Appendix C.2 and Figure 9).

Three cases of hypercalcaemia and three cases of hypercalciuria in 6 different children were reported in Lewis et al. (2013). However, these cases did not appear to be related to the dose of vitamin D administered, as none occurred in the highest dose arm of 100 μg/day. In Maalouf et al. (2008), five cases of hypercalcaemia were reported in the placebo arm while one case was reported in both the 5 and 50 μg/day vitamin D_3 intervention arms. It was unclear whether the reported hypercalcaemia cases in these two RCTs were persistent as there was no mentioning of retesting. One RCT (Asghari et al., 2021) that did not define cut-off values for hypercalcaemia reported interquartile ranges of serum calcium concentrations from 2.50 to 2.675 at 6 months and from 2.53 to 2.68 mmol/L at 12 months across treatment groups (i.e., 15, 25 and 50 μg/day), suggesting that some individuals may have developed hypercalcaemia. However, the data available do not allow assessing whether such potential cases were specifically related to the vitamin D dose given. None of the other studies reported any cases of hypercalcaemia Figure 9.

The overall RoB for all the six RCTs was considered moderate (Tier 2). The study by Lewis et al. (2013) was rated Tier 2 also for hypercalciuria. All three key domains raised concerns, in particular in relation to the outcome assessment (n = 4) and the characterisation of the exposure (n = 3). The heat map can be found in Appendix B.2. See also Annex J of the technical report for detailed justification of the RoB appraisal per domain for each study (Lamberg-Allardt et al., 2023).

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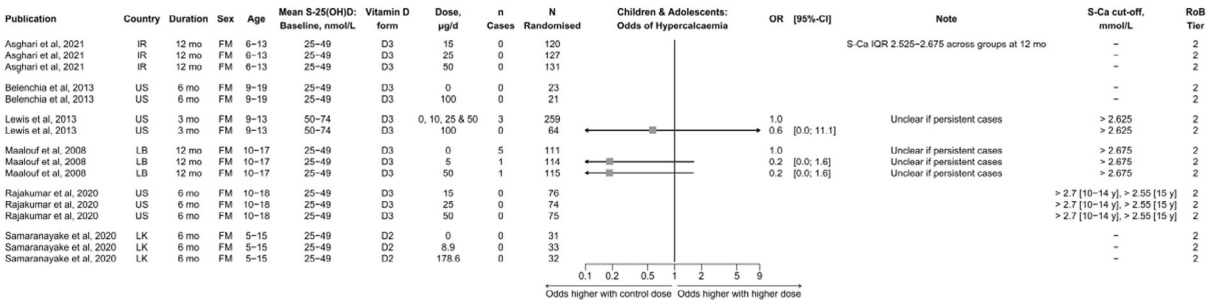

Figure 9: The effect of high doses of vitamin D on the odds of developing persistent hypercalcaemia in children and adolescents

For age, recruitment target range is presented. Mean baseline S-25(OH)D concentrations were classified as follows: < 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, ≥ 75 nmol/L. 'Cases' indicates the number of participants who developed hypercalcaemia. All effect sizes were estimated because pre-calculated effect sizes were not available.
Abbreviations: BMI, body mass index; F, females; IQR, inter quartile range; IR, Iran; LB, Lebanon; LK, Sri Lanka; M, males; S-25(OH)D, serum 25-hydroxyvitamin D; S-Ca, serum calcium; OR, odds ratio; RoB, risk of bias; US, United States.

The Panel considers that, in the available RCTs, the intake of vitamin D supplements at doses up to 179 µg/day for 3–12 months did not increase the risk of persistent hypercalcaemia or hypercalciuria in children and adolescents aged 5–18 years as compared to lower control doses or placebo. However, the Panel notes that, among the six RCTs investigating doses of vitamin D ≥ 50 µg/day, only one reports on the risk of hypercalciuria, four recruited exclusively overweight/obese individuals with baseline serum 25(OH)D concentrations < 50 nmol/L, none were conducted in Europe, and all were of moderate RoB (Tier 2).

3.5.1.2. Pregnant and lactating women

Five RCTs conducted in pregnant or lactating women investigated the effects of vitamin D supplementation on hypercalcaemia, while three also reported on the urinary calcium to creatinine (U-Ca/Cr) ratio, a reliable indicator for monitoring hypercalciuria (Hollis and Wagner, 2004; Wagner et al., 2006; Hollis et al., 2011).

The studies were conducted in Bangladesh, Mongolia, and the USA. No studies were conducted in Europe and Caucasian populations were underrepresented. The intervention lasted 6 months in the studies including only pregnant women (Hollis et al., 2011; Enkhmaa et al., 2019), three (Hollis and Wagner, 2004) or six months (Wagner et al., 2006) in those including only lactating women, and from 17 to 24 weeks of gestation to 26 weeks postpartum in the study in pregnant and lactating women (Roth et al., 2018). Baseline mean serum 25(OH)D concentrations widely varied across studies, from < 25 nmol/L (Enkhmaa et al., 2019) to > 75 nmol/L (Hollis and Wagner, 2004; Wagner et al., 2006). Four RCTs administered vitamin D_3 supplements and one a combination of D_2 and D_3 (Hollis and Wagner, 2004). Two RCTs used calcium as a co-intervention. The highest vitamin D dose administered was 100 µg/day in four studies and 160 µg/day in one (see Appendix C.2 for the evidence table and Figure 10).

One RCT used a serum calcium cut-off point of 2.60 mmol/L for hypercalcaemia (Enkhmaa et al., 2019) and one defined confirmed hypercalcaemia as a serum calcium concentration above 2.60 mmol/L on a repeat test or as a single serum calcium concentration above 2.80 mmol/L (Roth et al., 2018). In Hollis et al. (2011), provisional urinary Ca/Cr ratios ≥ 0.8 mg/mg for case review and ≥ 1 mg/mg for stopping vitamin D supplementation were set. No cut-off points for defining hypercalcaemia or hypercalciuria were provided in the remaining studies.

No cases of hypercalcaemia were reported in pregnant or lactating women at vitamin D doses of 100 µg/day in three RCTs (Hollis and Wagner, 2004; Wagner et al., 2006; Enkhmaa et al., 2019). In the RCT by Hollis et al. (2011) the authors report that no significant differences were found between groups on any safety measure, including serum calcium, and that no single adverse event could be attributed to vitamin D supplementation.

In Roth et al. (2018), no cases of hypercalcaemia were observed during the prenatal period. However, 5 women (1.9%) who received 100 µg/day vitamin D_3 and one woman (0.4%) in the placebo group developed hypercalcaemia postpartum (Figure 10a). In addition to the placebo and

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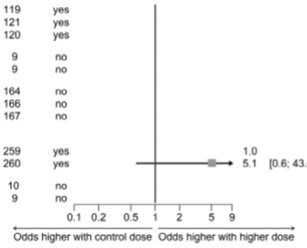
100 μg/day intervention arms that were investigated for the whole duration of the study, three additional arms receiving 15, 60 and 100 μg/day vitamin D_3 for the prenatal period and placebo after delivery were also investigated (not included in Figure 10a). One participant each in the 60 μg/day and the 100 μg/day groups developed hypercalcaemia after childbirth when taking placebo (Roth et al., 2018). As hypercalcaemia could be confirmed with either a repeat or a single serum calcium measurement, it is unclear whether the reported cases were persistent.

No cases of persistent hypercalciuria were reported (Figure 10b). In Hollis et al. (2011), the only case in which supplementation was stopped was due to elevated serum 25(OH)D concentrations, with normal urinary Ca/Cr ratio, suggesting that no women had a urinary Ca/Cr ratio ≥ 1 mg/mg in any group.

The overall RoB was considered moderate (Tier 2) for hypercalcaemia (5 RCTs) and hypercalciuria (3 RCTs), critical domains being outcome assessment and attrition. The heat map can be found in Appendix B.2. See also Annex J and K of the technical report for detailed justification for the RoB appraisal per domain for each study (Lamberg-Allardt et al., 2023).

(a)

Publication	Country	Duration	Status	Age	Mean S-25(OH)D: Baseline, nmol/L	Vitamin D form	Dose, μg/d	n Cases	N Randomised	Ca	Pregnant & Lactating women: Odds of Hypercalcaemia	OR	[95%-CI]	Note	S-Ca cut-off, mmol/L	RoB Tier
Enkhmaa et al, 2019	MN	24 wk	pregnant	≥ 18	< 25	D3	15	0	119	yes					> 2.60	2
Enkhmaa et al, 2019	MN	24 wk	pregnant	≥ 18	< 25	D3	50	0	121	yes					> 2.60	2
Enkhmaa et al, 2019	MN	24 wk	pregnant	≥ 18	< 25	D3	100	0	120	yes					> 2.60	2
Hollis & Wagner, 2004	US	3 mo	lactating	29 [mean]	50–74	D2 + D3	50	0	9	no					–	2
Hollis & Wagner, 2004	US	3 mo	lactating	31 [mean]	≥ 75	D2 + D3	100	0	9	no					–	2
Hollis et al, 2011	US	*	pregnant	27 [mean]	50–74	D3	10	NR	164	no				Case numbers by group NR	–	2
Hollis et al, 2011	US	*	pregnant	27 [mean]	50–74	D3	50	NR	166	no				Case numbers by group NR	–	2
Hollis et al, 2011	US	*	pregnant	27 [mean]	50–74	D3	100	NR	167	no				Case numbers by group NR	–	2
MDIG																
Roth et al, 2018	BD	**	pregnant and lactating	≥ 18	25–49	D3	0	1	259	yes		1.0		or > 2.80 in single specimen	> 2.60	2
Roth et al, 2018	BD	**	pregnant and lactating	≥ 18	25–49	D3	100	5	260	yes		5.1	[0.6; 43.6]	or > 2.80 in single specimen	> 2.60	2
Wagner et al, 2006	US	6 mo	lactating	30 [mean]	≥ 75	D3	10	0	10	no					–	2
Wagner et al, 2006	US	6 mo	lactating	28 [mean]	≥ 75	D3	160	0	9	no					–	2



(b)

Publication	Country	Duration	Status	Age	Mean S-25(OH)D: Baseline, nmol/L	Vitamin D form	Dose, μg/d	n Cases	N Randomised	Ca	Pregnant & Lactating women: Odds of Hypercalciuria	OR	[95%-CI]	Note	U-Ca cut-off	RoB Tier
Hollis & Wagner, 2004	US	3 mo	lactating	29 [mean]	50–74	D2 + D3	50	0	9	no					–	2
Hollis & Wagner, 2004	US	3 mo	lactating	31 [mean]	≥ 75	D2 + D3	100	0	9	no					–	2
Hollis et al, 2011	US	*	pregnant	27 [mean]	50–74	D3	10	NR	164	no				Case numbers by group NR	–	2
Hollis et al, 2011	US	*	pregnant	27 [mean]	50–74	D3	50	NR	166	no				Case numbers by group NR	–	2
Hollis et al, 2011	US	*	pregnant	27 [mean]	50–74	D3	100	NR	167	no				Case numbers by group NR	–	2
Wagner et al, 2006	US	6 mo	lactating	30 [mean]	≥ 75	D3	10	0	10	no					–	2
Wagner et al, 2006	US	6 mo	lactating	28 [mean]	≥ 75	D3	160	0	9	no					–	2

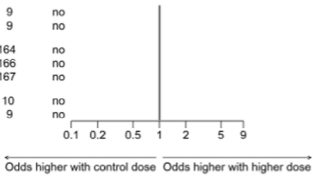


Figure 10: The effect of high doses of vitamin D on the odds of developing persistent (a) hypercalcaemia or (b) hypercalciuria in pregnant and lactating women

For age, recruitment target range is presented. Mean baseline S-25(OH)D concentrations were classified as follows: < 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, ≥ 75 nmol/L. 'Cases' indicates the number of participants who developed hypercalcaemia or hypercalciuria. 'Ca' indicates whether calcium was provided as a co-intervention. **Abbreviations:** BD, Bangladesh; BMI, body mass index; MDIG, Maternal Vitamin D for Infant Growth; MN, Mongolia; NR, not reported; OR, odds ratio; RoB, risk of bias; S-25(OH)D, serum 25-hydroxyvitamin D; S-Ca, serum calcium; U-Ca, urinary calcium; US, United States. **Note:** In the RCT of Hollis and Wagner (2004), 100 μg/day of vitamin D (90 μg/day of vitamin D_2 + 10 μg/day of vitamin D_3) was compared with 50 μg/day of vitamin D (40 μg/day of vitamin D_2 + 10 μg/day of vitamin D_3). Effect sizes for Roth et al., (2018) were estimated because pre-calculated effect sizes were not available.

The Panel considers that, in the available RCTs, the intake of vitamin D supplements at doses up to 160 μg/day consumed for 4–6 months during pregnancy or lactation did not increase the risk of persistent hypercalcaemia or hypercalciuria in pregnant or lactating women as compared to lower control doses or placebo. The Panel also notes that, among the five RCTs investigating doses of vitamin D ≥ 100 μg/day, the risk of hypercalcaemia post-partum was higher in the group consuming vitamin D at 100 μg/day versus placebo in the only study providing vitamin D in co-supplementation with calcium during pregnancy and lactation (Roth et al., 2018), although it is unclear whether the reported cases were persistent. The Panel also notes the paucity of data available, that none of the available RCTs were conducted in Europe and that they were at moderate RoB.

3.5.1.3. Adults

A total of 23 RCTs reporting on hypercalcaemia were conducted in adults. These are presented in two separate categories based on the duration of the supplementation (≤ 6 months, 'short-term',

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n = 13; ≥ 1 year, 'long-term', n = 10). Ten of these RCTs also reported on hypercalciuria, whereas one additional RCT reported on hypercalciuria alone. The 11 RCTs reporting on hypercalciuria are presented together.

Hypercalcaemia

RCTs with an intervention period ≤ 6 months

Of the 13 RCTs available, eight RCTs were conducted in USA (Heaney et al., 2003; Burnett-Bowie et al., 2012; Ponda et al., 2012; Aloia et al., 2013; Drincic et al., 2013; Rorie et al., 2014; Schwartz et al., 2016; Shirvani et al., 2020), two in Europe (Wamberg et al., 2013, 2016), one in Canada (Vieth et al., 2001), one in Australia (Diamond et al., 2013), and one in Argentina (Mastaglia et al., 2006).

Two RCTs included only females, one included only men (Heaney et al., 2003) while the other 10 RCTs included both sexes. In most studies (n = 9) participants were overweight or obese on average. The mean baseline serum 25(OH)D concentrations were between 25 and 49 nmol/L in eight RCTs, between 50 to 74 nmol/L in five RCTs, and ≥ 75 nmol/L in one RCT (Rorie et al., 2014).

Two RCTs administered vitamin D_2 and 11 gave vitamin D_3 as supplements. The highest vitamin D dose administered ranged from 100 to 275 μg/day (Heaney et al., 2003). Three RCTs used calcium as a co-intervention. Serum calcium (or ionised, free calcium) concentration cut-off points for determining hypercalcaemia differed across the 8 RCTs which specified those values. Serum calcium cut-offs ranged from 2.575 to 2.75 mmol/L (n = 6) and ionised calcium cut-offs were 1.32 and 1.35 (n = 2). See Appendix C.2 and Figure 11a for details on the characteristics of the studies.

Of the available studies, six had included participants with baseline serum 25(OH)D < 50 nmol/L and eight had small sample sizes (between 8 and 24 participants per arm). None of the available RCTs reported cases of persistent hypercalcaemia (see Table 8 for criteria) with vitamin D doses of up to 275 μg/day (Figure 11a). One RCT reported one transient case of hypercalcaemia in the arm supplemented with 100 μg/day of vitamin D_3 and 1,200 mg/day of calcium, whereas no cases were observed with supplementation of vitamin D alone, calcium alone, or placebo over the 6-month intervention period (Aloia et al., 2013). Similarly, in Schwartz et al. (2016), one case of hypercalcaemia was reported in the 100 μg/day vitamin D_3 arm, which resolved after the participant stopped taking spontaneous supplemental calcium (not planned as co-intervention). Another RCT (Wamberg et al., 2013) reported one subject in the 175 μg/day of vitamin D_3 arm being just above the upper limit for hypercalcaemia on one occasion, which cannot be considered as persistent.

Studies with an intervention period ≥ 1 year

Of the 10 RCTs available, five were conducted in USA (Gallagher et al., 2012; Gallagher et al., 2013; Aloia et al., 2018; Rafii et al., 2019; Johnson et al., 2022), four in Europe (Brohult and Jonson, 1973; Sneve et al., 2008; Grimnes et al., 2012; Hin et al., 2016) and one in Canada (Billington et al., 2020). Four RCTs were performed in females only, while the other six RCTs included both sexes. In eight studies, participants were overweight or obese. Mean serum 25(OH)D concentrations at baseline were between 25 and 49 nmol/L in two RCTs, between 50 and 74 nmol/L in five RCTs, and ≥ 75 nmol/L in one (Billington et al., 2020), while they ranged from 47 to 55 nmol/L across the study arms in one RCT (Hin et al., 2016). One RCT did not report baseline 25(OH)D concentrations (Brohult and Jonson, 1973).

With the exception of Rafii et al. (2019), which investigated both vitamin D_2 and D_3, the remaining studies used vitamin D_3 supplements. Seven RCTs had used a calcium co-intervention. The highest vitamin D dose administered ranged from 100 μg/day (Hin et al., 2016; Johnson et al., 2022) to 357 μg/day (Rafii et al., 2019), and up to 2,500 μg/day in an old study (Brohult and Jonson, 1973). The duration of the intervention ranged from 1 to 3 years.

The criteria for defining hypercalcaemia varied across the RCTs. Cut-off points ranged between 2.55 and 2.8 mmol/L across the studies (n = 8). One study did not define hypercalcaemia (Brohult and Jonson, 1973). Johnson et al. (2022) defined hypercalcaemia as serum calcium levels 'greater than the site's clinical laboratory upper level of normal plus 0.25 mmol/L, where no repeat test was needed, or a repeat test that showed values greater than the site's clinical laboratory upper level of normal'. Overall, seven of the 10 RCTs reported remeasuring serum calcium concentrations when hypercalcaemia was observed. See Appendix C.2 and Figure 11b for details on the characteristics of the studies.

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Out of 10 RCTs, three reported cases of persistent hypercalcaemia with varying doses of vitamin D_3 (i.e., 71 to 250 μg/day), but the occurrence was rare and not specifically related to the dose of vitamin D_3 (Figure 11b). In a 3-year RCT (Johnson et al., 2022), daily supplementation with 100 μg of vitamin D_3 resulted in an incidence rate ratio (IRR) of 1.49 (95% CI, 0.42, 5.28) compared to placebo in a large sample of prediabetic and obese individuals with baseline mean serum 25(OH)D $>$ 50 nmol/L. Of the 20 cases in the vitamin D_3 group and 16 cases in the placebo group identified as suspect hypercalcaemia cases, 6 and 4 cases, respectively, were confirmed as persistent on repeated measurements. Over the 3-year period, 27 cases of nephrolithiasis in the vitamin D_3 group and 23 in the placebo group were self-reported and subsequently adjudicated by the investigators when medical records were available for review. About 1/3 of participants consumed calcium supplements up to 600 mg/day. In another 3-year RCT in subjects with mean serum 25(OH)D concentrations at baseline $\geq$ 75 nmol/L, Billington et al. (2020) found that one participant who received 250 μg/day of vitamin D_3 in co-supplementation with calcium (target 1,200 mg/day from diet and supplements) developed transient hypercalcaemia twice, at 6 and 30 months. This was considered as a persistent case. The study also found that the frequency of non-recurrent, transient hypercalcaemia increased with higher doses of vitamin D_3, with 4 cases reported with 100 μg/day, 12 cases with 250 μg/day, and none with 10 μg/day ($p = 0.02$). Hypercalcaemia events were mild and resolved on follow-up testing, before which calcium intake was reduced in 10 cases. In the third study (Sneve et al., 2008), one case of hypercalcaemia was also identified over the one-year study period in overweight or obese individuals treated with 71 μg/day of vitamin D_3, with no cases reported in the placebo group or the 143 μg/day vitamin D_3 arm. All groups received 500 mg/day of supplemental calcium. The Panel notes that none of the RCTs reported cases of persistent hypercalcaemia (see Table 8 for criteria) that could be specifically attributed to the dose of vitamin D used for supplementation.

Two RCTs reported the occurrence of hypercalcaemia cases (unclear if persistent) with doses of 250 and 2,500 μg/day vitamin D_3 over one year (Figure 11b). Brohult and Jonson (1973) noted that in a sample of patients with rheumatoid arthritis, one participant assigned to 2,500 μg/day of vitamin D_3 had elevated serum calcium levels at 10 months but did not mention retesting. No cases occurred in the placebo group. The other RCT (Aloia et al., 2018) reported that for hypercalcaemia computed after correcting serum calcium for albumin, 21% of participants (14/66 patients, 20 hypercalcaemic events) had developed hypercalcaemia at least once with vitamin D_3 at 250 μg/day co-administered with calcium supplements (1,200 mg/day), in comparison to 17% of participants (11/66 patients, 17 hypercalcaemic events) in the lower vitamin D dose group (15 μg/day plus 1,200 mg/day of calcium). The crude OR (95CI%) for developing hypercalcaemia in 250 μg/day versus the 15 μg/day group was 1.3 (0.6, 3.2). The authors noted that the occurrence of hypercalcaemia over time was not different in the high versus the low dose group (Aloia et al., 2018).

Three RCTs reported cases of transient hypercalcaemia with vitamin D doses of 100, 120 and 163 μg/day, with no clear evidence of a dose–response relationship. In one study conducted in obese postmenopausal women with mean serum 25(OH)D $<$ 50 nmol/L at baseline, cases of hypercalcaemia were temporary and normalised at repeated testing (Gallagher et al., 2012). In the other study conducted in postmenopausal women with serum 25(OH)D at baseline $\sim$ 71 nmol/L, modest hypercalcaemia (serum calcium 2.60–2.80 mmol/L) occurred at serum 25(OH)D concentrations between 64 and 256 nmol/L, but all cases had resolved at retesting (Grimnes et al., 2012). There was no significant difference in the number of participants that had serum calcium levels $\geq$ 2.60 mmol/L, with 9 cases in the 163 μg/day arm and 4 cases in the 20 μg/day arm. No cases of severe hypercalcaemia, defined as serum calcium $>$ 2.80 mmol/L, were reported. Another 1-year study that investigated various vitamin D doses of up to 120 μg/day reported the occurrence of hypercalcaemia in 7% of participants, with a total of 12 cases, without specifying the group these cases occurred in (Gallagher et al., 2013). The authors did report there was no correlation between vitamin D dose and hypercalcaemia. Only one participant (from the placebo group) discontinued the intervention due to persistent hypercalcaemia, as per study protocol.

No cases of hypercalcaemia, as defined by the authors, were observed in the other two RCTs during the 1-year interventions with vitamin D doses between 50 and 375 μg/day (Hin et al., 2016; Rafii et al., 2019).

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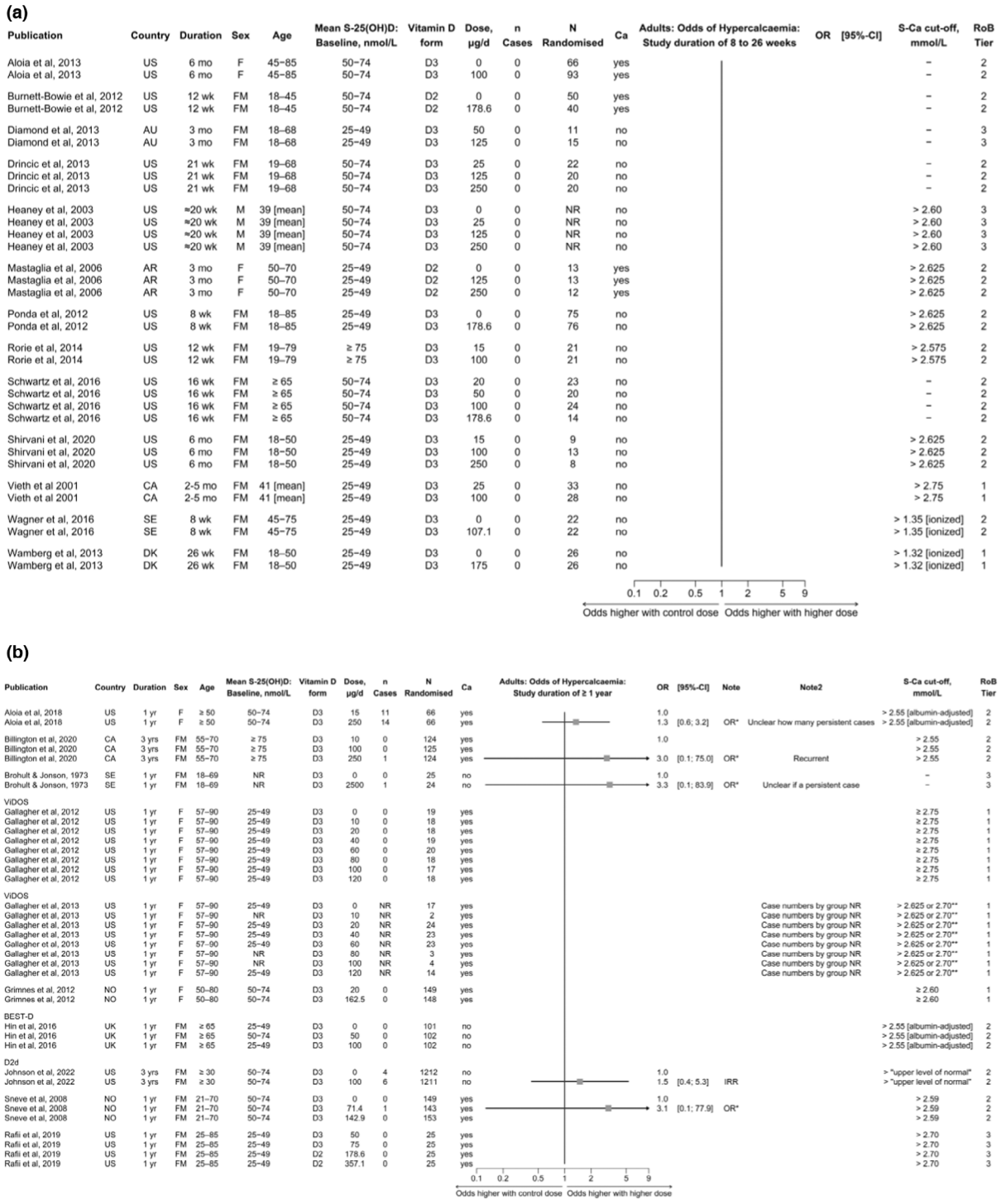

Figure 11: The effect of high doses of vitamin D on the odds of developing persistent hypercalcaemia among general adult populations treated with vitamin D for (a) 8–26 weeks and (b) 1 year or more

For age, recruitment target range is presented. Mean baseline S-25(OH)D concentrations were classified as follows: < 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, ≥ 75 nmol/L. 'Cases' indicates the number of participants who developed hypercalcaemia. 'Ca' indicates whether calcium was provided as a co-intervention. 'Note' indicate the effect size included in the forest plot. Pre-calculated effect sizes were used if available without performing conversions; please note that effect sizes can be therefore different from the one expressed in the plot title. 'Note2' provides further information on the outcome. 'Recurrent' refers to the number of participants who demonstrated elevated S-Ca at least at two measurement points after baseline. The zero cases refer to no

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occurrence of hypercalcaemia or to transient hypercalcaemia. **Abbreviations:** AR, Argentina; AU, Australia; BEST-D: Biochemical Efficacy and Safety Trial of vitamin D; CA, Canada; CI, confidence interval; D2d: the Vitamin D and Type 2 Diabetes; DK, Denmark; F, females; M, males; NO, Norway; NR, not reported; S-25(OH)D, serum 25-hydroxyvitamin D; S-Ca, serum calcium; SE, Sweden; OR, odds ratio; RoB, risk of bias; UK, United Kingdom; US, United States; ViDOS, Vitamin D supplementation in Older Women. **Note:** Aloia et al. (2013) used a 2 × 2 factorial design, but the results are shown for the groups with vitamin D and without vitamin D.

*The effect size was computed based on case numbers because pre-calculated effect sizes were not available;

**Two study sites applied different cut-off points.

The overall RoB for trials assessing hypercalcaemia in adults was considered low (Tier 1) for five studies, moderate (Tier 2) for 14 studies and high (Tier 3) for four studies. Critical domains were exposure characterisation (n = 9), outcome assessment (n = 13), allocation concealment (n = 11), blinding (n = 8) and attrition (n = 10) (see heat map in Appendix B.2). See also Annex J of the external technical report for justification of the RoB appraisal (Lamberg-Allardt et al., 2023).

The Panel notes that the cases of persistent hypercalcaemia reported in three of the available RCTs at supplemental doses of vitamin D up to 250 μg/day may not be specifically attributed to the dose of vitamin D used for supplementation (Sneve et al., 2008; Billington et al., 2020; Johnson et al., 2022), and that such cases occurred with concurrent calcium supplementation (either personal use or prescribed in the study). These studies were at moderate RoB (tier 2). Cases of transient hypercalcaemia were also mostly observed with calcium co-supplementation and resolved with calcium withdrawal. In a RCT at high RoB (Brohult and Jonson, 1973), one case of hypercalcaemia (unclear if persistent) was observed with vitamin D supplementation alone at doses of 2,500 μg/day consumed for one year. The Panel also notes that the three RCTs of small sample size (8–20 subjects per arm) which provided vitamin D_3 supplementation alone at doses of 250–275 μg/day for 5–6 months did not report any cases of hypercalcaemia ((Heaney et al., 2003), RoB tier 3;(Drincic et al., 2013; Shirvani et al., 2020), RoB tier 2).

Hypercalciuria

A total of 11 RCTs were identified reporting on hypercalciuria, 10 of which have already been assessed in relation to hypercalcaemia and one measured hypercalciuria alone (Ceglia et al., 2013).

All the RCTs were conducted in America: eight in the USA (Gallagher et al., 2012; Aloia et al., 2013; Ceglia et al., 2013; Gallagher et al., 2013; Rorie et al., 2014; Aloia et al., 2018; Rafii et al., 2019; Johnson et al., 2022), two in Canada (Vieth et al., 2001; Billington et al., 2020) and one in Argentina (Mastaglia et al., 2006). Six RCTs were conducted in postmenopausal females and five included both sexes. In six studies, participants were mostly overweight or obese (Gallagher et al., 2012; Gallagher et al., 2013; Rorie et al., 2014; Aloia et al., 2018; Billington et al., 2020; Johnson et al., 2022). Mean baseline serum 25(OH)D concentrations were between 25 and 49 nmol/L in five RCTs and between 50 to 74 nmol/L in four RCTs, while the mean concentrations were ≥ 75 nmol/L in two RCTs (Rorie et al., 2014; Billington et al., 2020).

Two RCTs used vitamin D_2 and the remaining vitamin D_3. The highest dose of vitamin D administered on each trial ranged from 100 to 250 μg/day (Mastaglia et al., 2006; Aloia et al., 2018; Billington et al., 2020) and up to 357 μg/day (Rafii et al., 2019), and the duration of the intervention ranged from 2.5 months (Vieth et al., 2001) to three years (Billington et al., 2020; Johnson et al., 2022). Seven RCTs reported the use of calcium supplements as co-intervention.

Five RCTs used cut-offs for urinary calcium in 24-h urine collections to define hypercalciuria, which were variable and ranged between > 6.25 and up to >7.5 mmol. In the remaining RCTs (Figure 12), hypercalciuria was defined using very heterogeneous cut-offs of the urinary calcium/creatinine (Ca/Cr) ratio assessed in spot urine (n = 5), ranging from 0.395 to 1.06 on molar basis, or calcium levels in spot urine > 7.49 mmol/L (Rorie et al., 2014). In one RCT (Gallagher et al., 2012), to account for the potential error of urine overcollection, episodes of hypercalciuria were excluded from the analysis when the 24-h urine creatinine level was > 20% the mean of all collections combined.

See Appendix C.2 and Figure 12 for details on the characteristics of the studies and section 3.3.1.4 of the external technical report for thorough description of the individual studies.

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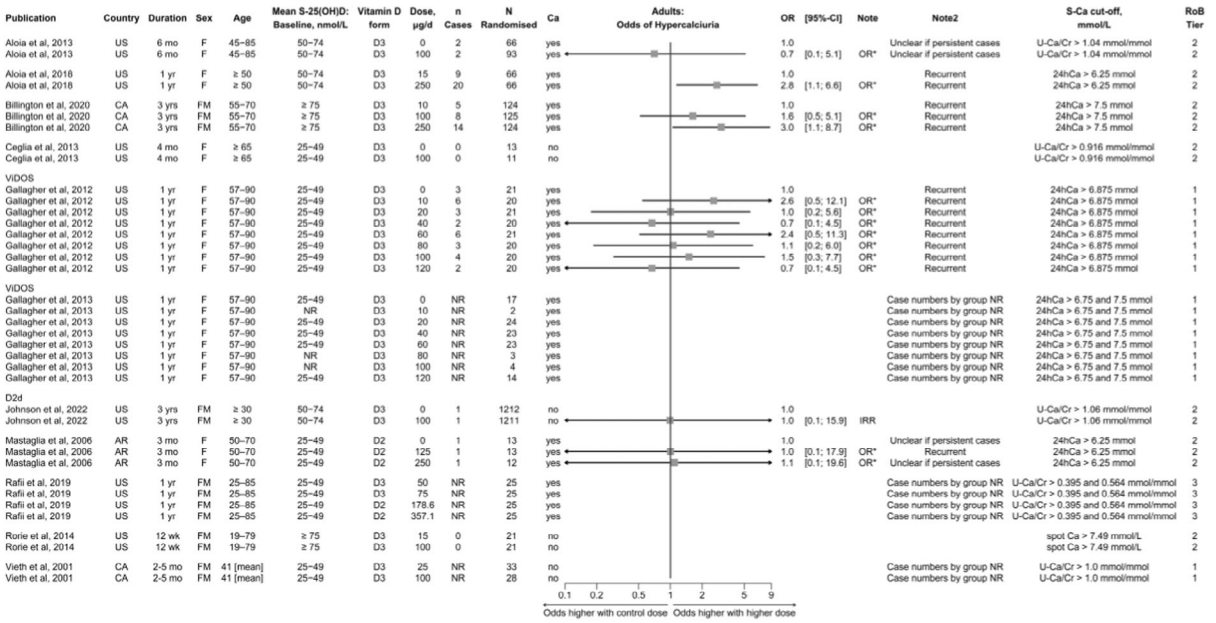

Figure 12: The effect of high doses of vitamin D on the odds of developing persistent hypercalciuria among general adult populations

For age, recruitment target range is presented. Mean baseline S-25(OH)D concentrations were classified as follows: < 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, ≥ 75 nmol/L. 'Cases' indicates the number of participants who developed hypercalciuria. 'Ca' indicates whether calcium was provided as a co-intervention. 'Note' indicates the effect size included in the forest plot. Pre-calculated effect sizes were used if available, without performing conversions; please note that effect sizes can be therefore different from the one expressed in the plot title. 'Note2' provides further information on the outcome. 'Recurrent' refers to the number of participants who demonstrated elevated U-Ca at least at two measurement points after baseline. The zero cases refer to no occurrence of hypercalciuria or to transient hypercalciuria. **Abbreviations:** AR, Argentina; CA, Canada; Ca, calcium; CI, confidence interval; F, females; IRR, incidence rate ratio; M, males; NR, not reported; OR, odds ratio; Rob, risk of bias; S-25(OH)D, serum 25-hydroxyvitamin D; U-Ca, urinary calcium; U-Ca/Cr, urinary calcium/creatinine ratio; US, United States. **Note:** Recurrent hypercalciuria cases for Gallagher et al. (2012, 2014b) were received from prof. Gallagher (personal communication).

*The effect size was computed based on case numbers because pre-calculated effect sizes were not available.

The OR for hypercalciuria could not be calculated for five RCTs, either because no cases of persistent hypercalciuria were identified (highest dose of vitamin D tested 100 µg/day; [Ceglia et al., 2013; Rorie et al., 2014]) or because the number of cases per intervention arm was not reported (Vieth et al., 2001; Gallagher et al., 2013; Rafii et al., 2019). In the latter 3 RCTs (highest doses tested between 100 and 357 µg/day of vitamin D_3 and 75 µg/day of vitamin D_2; two RCTs in co-supplementation with calcium), cases of hypercalciuria (unclear if persistent) were reported to be not significantly different across intervention arms (Figure 12).

Two RCTs reported the occurrence of hypercalciuria with 100 µg/day of vitamin D_3 (Aloia et al., 2013), and with 125 and 250 µg/day of vitamin D_2 (Mastaglia et al., 2006) in co-supplementation with calcium. However, it was unclear whether these cases were all persistent and the occurrence of hypercalciuria was not dose-dependent (i.e., same number of cases in all study arms; Figure 12) (Mastaglia et al., 2006; Aloia et al., 2013).

The remaining four RCTs reported on the occurrence of persistent hypercalciuria at doses of vitamin D_3 ranging from 10 to 250 µg/day among participants who were overweight or obese on average (Gallagher et al., 2012; Gallagher et al., 2013; Aloia et al., 2018; Billington et al., 2020; Johnson et al., 2022). In two RCTs the occurrence of recurrent hypercalciuria was unrelated to the dose of vitamin D used, which ranged from 10 to 120 µg/day in Gallagher et al. (2012) (five vitamin D_3 intervention arms in co-supplementation with calcium) and was 100 µg/day in Johnson et al. (2022). In the remaining two RCTs, the odds of developing recurrent hypercalciuria was approximately three times higher with 250 µg/day of vitamin D_3 than with control doses during the

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intervention period of 1 year (Aloia et al., 2018) or 3 years (Billington et al., 2020). In both RCTs vitamin D_3 was provided in co-supplementation with calcium and participants had mean serum 25(OH) D of 69 to 70 nmol/L (Aloia et al., 2018) and 76 to 80 nmol/L across arms (Billington et al., 2020).

In the RCT by Aloia et al. (2018), doses of 15 and 250 μg/day of vitamin D_3 were provided for 1 year together with calcium (1,200 mg/day) to healthy Caucasian post-menopausal women (n = 66 per arm). Calcium supplements were reduced to 600 mg/day if hypercalcaemia or hypercalciuria episodes were confirmed on repeated measures and removed completely if the episodes persisted on follow-up visits. Persistent hypercalcaemia or hypercalciuria after calcium withdrawal would lead to vitamin D supplements discontinuation. In the 250 μg/day vitamin D_3 group, hypercalciuria was detected in 34 subjects (14, 15 and 5 subjects developed hypercalciuria once, 2–3 times and 4 times during the study, respectively) versus 19 subjects (10, 7 and 2 subjects developed hypercalciuria once, 2–3 times and 4 times during the course of the study, respectively) in the 15 μg/day vitamin D_3 group. Among subjects with hypercalciuria, 6 and 4 in the 250 μg/day group and 2 and 0 in the 15 μg/day group using serum calcium cut-offs of $>$ 10.2 and $>$ 10.5 mg/dL, respectively, had hypercalcaemia when hypercalciuria was detected. The crude OR (95% CI) based on the number of cases per group for recurrent hypercalciuria was 2.8 (1.1, 6.6) (Figure 12). Subjects with persistent hypercalciuria had discontinued calcium supplements at the end of the study. The authors noted that the occurrence of hypercalciuria over time was significantly higher in the high versus the low dose group, and that calcium intake was statistically significant when included as a time-varying covariate in the model. Mean (SD) achieved serum 25(OH)D concentrations in the 250 μg/day and the 15 μg/day groups were 216 nmol/L (66 nmol/L) and 84.3 nmol/L (15.3 nmol/L), respectively. This study was at moderate RoB (Tier 2), critical domains being allocation concealment, blinding and attrition.

In the other RCT (Billington et al., 2020) healthy adults of both sexes aged 55 to 70 years were randomised to consume supplemental vitamin D_3 at doses of 10, 100 or 250 μg/day and calcium supplements when dietary intake was $<$ 1,200 mg/day to achieve that level of intake (n $\approx$ 124 per arm). Participants were asked to reduce calcium supplements and/or dietary calcium when episodes of hypercalciuria or hypercalcaemia were detected. A urinary Ca/Cr ratio of 1.0 mmol/mmol or more conducted at follow-up of an elevated 24-h urine calcium excretion resulted in discontinuation of the study treatment. Hypercalciuria was observed in 4.3% of participants at baseline. At least one episode of hypercalciuria occurred in 21 (16.9%), 28 (22.4%), and 38 (30.6%) participants at doses of 10, 100 or 250 μg/day. Of these, only 5 (4.0%), 8 (6.4%), and 14 (11.3%) were recurrent, suggesting a dose–response relationship. The OR (95% CI) versus the 10 μg/day dose was 1.6 (0.5, 5.1) for the 100 μg/day dose and 3.0 (1.1, 8.7) for the 250 μg/day dose. No participants discontinued the study treatment because of hypercalciuria. Mean serum 25(OH)D concentrations were significantly higher (137 vs. 121 nmol/L) and PTH concentrations significantly lower (17.1 vs. 20.2 ng/L) during hypercalciuria episodes as compared to states of normocalciuria. Peak mean (standard deviation (SD)) serum 25(OH) D concentration achieved in the study by the 250 μg/day group was 198 (42) nmol/L, occurring at month 18. This study was at moderate RoB (Tier 2), critical domains being exposure characterisation (vitamin D was self-administered in drops, and the accuracy of drop content was not tested) and outcome assessment (no information was provided on the measurement of calcium in urine; the Ca/Cr ratio in spot urine was used if 24-h urinary calcium was not available).

The overall RoB for trials assessing hypercalciuria in adults was considered low (Tier 1) for three studies (Vieth et al., 2001; Gallagher et al., 2012; Gallagher et al., 2013), moderate (Tier 2) for seven studies and high (Tier 3) for one study (Rafii et al., 2019). Critical domains were randomization (n = 2), exposure characterisation (n = 3), outcome assessment (n = 5), allocation concealment (n = 3), blinding (n = 3) and attrition (n = 5) (see heat map in Appendix B.3). See also Annex K of the external technical report for justification of the RoB appraisal (Lamberg-Allardt et al., 2023).

The Panel notes that the available RCTs conducted with vitamin D_2 or D_3 supplements at doses up to 125 μg/day do not report cases of persistent hypercalciuria that can be specifically related to the vitamin D dose administered (i.e., the number of cases was similar across intervention arms, including placebo or lower vitamin D doses used as control), and occurred primarily with concomitant calcium supplementation. The Panel also notes, however, that two RCTs using daily doses of 250 μg/day vitamin D_3 in co-supplementation with calcium for one (Aloia et al., 2018) and 3 years (Billington et al., 2020) consistently report an increased risk of persistent hypercalciuria (about 3 times higher) in this supplementation arm as compared to lower vitamin D doses (10–15 μg/day), even when calcium supplements were reduced or withdrawn.

The Panel considers that the available RCTs suggest a positive relationship between vitamin D intake at doses of 250 μg/day for 1–3 years in co-supplementation with calcium and the risk of

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persistent hypercalciuria in adults. Cases of persistent hypercalciuria did not occur, or could not be specifically attributed to the vitamin D dose administered, when vitamin D supplements were given alone (up to 100 μg/day), at lower doses in combination with calcium (up to 125 μg/day), or for shorter periods of time (3–6 months). The Panel notes that most RCTs were at moderate RoB (Tier 2).

3.5.1.4. Mechanisms of toxicity

Calcium homeostasis is tightly regulated primarily by the action of two of the calciotropic hormones, namely PTH and $1,25(OH)_2D$ to maintain serum calcium concentrations within a narrow range of 2.25–2.60 mmol/L (ionised calcium 1.1–1.4 mmol/L). A decrease in extracellular ionised calcium increases secretion of PTH, which fosters calcium resorption and the conversion of 25(OH)D to $1,25(OH)_2D$ in the kidney, which in turn increases intestinal calcium absorption. Both PTH and $1,25(OH)_2D$ release calcium from bone stores through the activation of osteoclasts. All these processes contribute to the rise in extracellular ionised calcium. Conversely, when serum ionised calcium increases, PTH secretion is suppressed, leading to increased urinary calcium excretion, reduced intestinal calcium absorption and the inhibition of bone resorption. When the capacity threshold of the kidneys to excrete excess calcium is reached, serum ionised calcium concentrations start to increase, resulting in hypercalcaemia (Peacock, 2010).

A number of well described medical conditions can lead to hypercalcaemia owing to inappropriate secretion of PTH (hyperparathyroidism of various origin), excessive endogenous production of the active vitamin D metabolite $1,25(OH)_2D$ (e.g. congenital disorders, granulomatous diseases, lymphomas) or decreased inactivation (mutations of the CYP24A1 gene in children and adults). Exogenous vitamin D intoxication can also induce hypercalcaemia through increased bone resorption. Because of the prolonged half-life and accumulation of the 25(OH)D metabolite, the hypercalcaemic-hypercalciuric syndrome associated with vitamin D intoxication can persist for several weeks to months after treatment discontinuation, with an important morbidity and even extensive and permanent soft tissues damage by mineral deposits (Rizzoli, 2021).

In exogenous vitamin D-associated hypercalcaemia, serum 25(OH)D concentrations are increased, whereas serum concentrations of $1,25(OH)_2D$ are unchanged or even reduced, and serum PTH concentrations are appropriately reduced (EFSA NDA Panel, 2016; Tebben et al., 2016). In this context, some hypotheses for mechanisms of toxicity have been proposed. Based on *in vitro* radioligand binding assays with the VDR, it has been suggested that 25(OH)D at high concentrations in serum could bind the VDR in target tissues in sufficient amounts to induce processes that enhance intestinal calcium absorption and enhance bone mobilisation. Alternatively, the endogenous production of 5,6-trans-$25(OH)D_3$, a metabolite with higher affinity to the VDR, could contribute to vitamin D toxicity, but this metabolite has only been identified in animal models (Tebben et al., 2016). Another proposed mechanism of toxicity lays on the displacement of $1,25(OH)_2D$ from DBP by 25(OH)D or other metabolites, which could promote the entry of free $1,25(OH)_2D$ into target cells and bind the VDR (EFSA NDA Panel, 2018).

In general, serum total 25(OH)D concentrations $\geq$ 200–220 nmol/L are necessary to result in vitamin D toxicity (EFSA NDA Panel, 2012b, 2018). Serum 25(OH)D concentrations associated with hypercalcaemia, however, vary over a wide range (EFSA NDA Panel, 2012b) and are likely influenced by calcium intake. Hypercalcaemia is unlikely to occur with high intake of calcium from the diet alone but can be induced by high-dose calcium supplements, especially when accompanied by vitamin D supplements, as these can increase calcium absorption (EFSA NDA Panel, 2015). Indeed, together with increased bone resorption, absorption of dietary calcium by the intestine is a major factor contributing to hypercalcaemia in patients with high vitamin D intakes. The efficiency of calcium absorption depends on the amount of dietary calcium (inverse relationship) increases or decreases inversely, and adaptations to changes in calcium intake depend on $1,25(OH)_2D$. A decrease in calcium absorption efficiency of 0.21% per year after age 40 years and a one-time decrease of 2.2% with menopause have been described, possibly due to the development of resistance to the action of $1,25(OH)_2D$ and lower oestrogen levels affecting receptors in the small intestine, respectively (EFSA NDA Panel, 2015).

In summary, the mechanisms by which long-term, high oral intakes of vitamin D could induce persistent hypercalcaemia/hypercalciuria are not fully elucidated but plausible, validated pathways exist. However, the levels of exogenous vitamin D intake at which these adverse effects may occur are likely to be influenced by the amount of calcium intake and other individual factors affecting calcium and vitamin D metabolism. Hypercalciuria is likely to occur prior to hypercalcaemia owing to the tight homeostatic regulation of the latter.

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3.5.1.5. Evidence integration and uncertainty analysis

The UL for vitamin D for all population groups except infants (EFSA NDA Panel, 2018) derives from a NOAEL of 250 μg/day for hypercalcaemia in adults (EFSA NDA Panel, 2012b). The evidence available for the present assessment suggests, however, that persistent hypercalciuria may be an earlier sign of excess vitamin D than hypercalcaemia owing to the tight homeostatic regulation of the latter. Indeed, long-term supplementation (1–3 years) with vitamin D_3 at doses of 250 μg/day in co-supplementation with calcium increased the risk of persistent hypercalciuria about 3 times in adults (vs. lower control doses), whereas the same studies did not report such an increased risk for persistent hypercalcaemia ([Aloia et al., 2018; Billington et al., 2020]; RoB Tier 2).

Owing to the paucity of RCTs reporting cases of hypercalciuria per intervention arm in adults (n = 6) and, among them, the lack of RCTs available in the dose ranges between 15 and 100 μg/day and between 125 and 250 μg/day vitamin D, the Panel decided not to proceed with a quantitative evidence synthesis via dose–response analysis for this endpoint.

A limitation in the BoE is that less than half of the available RCTs available for the assessment of hypercalcaemia (n = 34) had monitored urine calcium levels (n = 14). In adults, of the 13 RCTs in which vitamin D was given alone at doses between the current UL (100 μg/day) and the current NOAEL (250 μg/day) for hypercalcaemia, which are the most appropriate to assess the adverse effects of vitamin D on these endpoints, only four assessed persistent hypercalciuria and the highest dose tested was 100 μg/day. The three RCTs that used vitamin D supplementation alone at doses of 250 μg/day reported no cases of hypercalcaemia but did not assess hypercalciuria (Heaney et al., 2003; Drincic et al., 2013; Shirvani et al., 2020).

A large body of evidence from RCTs in adults shows that oral vitamin D supplementation $<$ 100 μg/day, with or without calcium co-supplementation, does not lead to episodes of persistent hypercalcaemia or hypercalciuria that can be specifically attributed to the dose of vitamin D administered. This also applies to RCTs with vitamin D at 100 μg/day given alone (Vieth et al., 2001; Ceglia et al., 2013; Rorie et al., 2014; Johnson et al., 2022) or with calcium supplements (Gallagher et al., 2012; Aloia et al., 2013; Gallagher et al., 2013; Rafii et al., 2019). In the only RCT (Billington et al., 2020) suggesting a dose response relationship between vitamin D intake (in co-supplementation in calcium) and episodes of persistent hypercalciuria, these were not significantly different in the 100 μg/day versus the 10 μg/day vitamin D arms.

RCTs in children and adolescents, and in pregnant and lactating women, do not suggest a positive relationship between the intake of vitamin D supplements at doses up to 179 μg/day and 160 μg/day, respectively and the risk of persistent hypercalcaemia or hypercalciuria. However, the Panel notes the low number of RCTs available (particularly for hypercalciuria) at doses at or above the UL for these population groups (50 and 100 μg/day, respectively).

3.5.1.6. Conclusions on persistent hypercalcaemia and hypercalciuria

The Panel concludes, based on evidence from RCTs in adults, that:

a) vitamin D supplementation alone at doses up to 250 μg/day for 5–6 months does not increase the risk of persistent hypercalcaemia. It is important to note that persistent hypercalciuria was not assessed under these conditions;
b) supplementation with vitamin D at doses of 250 μg/day for a period of 1–3 years (in co-supplementation with calcium to reach adequate intakes for the study population) increases the risk of persistent hypercalciuria by approximately 3 times as compared to doses of vitamin D in the range of adequate intakes (10–15 μg/day), even when calcium supplementation was either reduced or withdrawn;
c) cases of persistent hypercalcaemia or persistent hypercalciuria did not occur or could not be specifically attributed to the dose of vitamin D when vitamin D supplements were given alone (up to a dose of 100 μg/day), or at doses up to 125 μg/day in combination with calcium, or for shorter periods of time (3–6 months).

Owing to the paucity of RCTs reporting cases of hypercalciuria per intervention arm in adults and the lack of RCTs available in the dose range between 125 and 250 μg/day vitamin D, no quantitative evidence synthesis via dose–response analysis for this endpoint was performed.

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3.5.2. Musculoskeletal health

A common literature search was undertaken for sQ4 on fractures (sQ4a), falls (sQ4b), BMD/BMC and indices of bone strength (sQ4c) because several studies report on combinations of these endpoints. However, different eligibility criteria applied for each endpoint (see Section 2.1.1.3 and Annex A of the opinion).

RCTs were eligible for falls and fractures and data was extracted from all the available RCTs identified. RCTs and prospective cohort studies (PCs) which assessed the relationship between vitamin D intakes or serum 25(OH)D concentrations and BMD/BMC were eligible. However, data from these PCs and RCTs investigating vitamin D doses $<$ 100 μg/day in adults and $<$ 50 μg/day in children were not extracted because no adverse effects on BMD/BMC or related indices of bone strength were reported (Section 2.1.1.3).

3.5.2.1. Bone fractures (sQ4a)

A total of eight RCTs that investigated the effect of vitamin D supplementation on risk of bone fractures in older adults for a period of at least one year were included in the assessment.

Four RCTs were conducted in Europe (Grant et al., 2005; Macdonald et al., 2013; Hin et al., 2016; Bischoff-Ferrari et al., 2020), one in Canada (Burt et al., 2019), two in Australia (Flicker et al., 2005; Prince et al., 2008) and one in the USA (Peacock et al., 2000) (Appendix C.3). Most participants were Caucasians. Two RCTs included females only (Prince et al., 2008; Macdonald et al., 2013) and the other six included both sexes. The mean age at baseline ranged between 62 and 84 years. Most participants were community dwelling individuals (n = 7 studies). One RCT recruited individuals with a history (previous 10 years) of low trauma osteoporotic fractures (Grant et al., 2005), one study recruited females with a history of falling in the past year (Prince et al., 2008), while another study reported recruiting $\geq$ 40% of participants with a history of falling in the prior 12 months (Bischoff-Ferrari et al., 2020). Mean baseline serum 25(OH)D concentrations ranged from 38 to 81.3 nmol/L across the intervention arms and one RCT reported that $\sim$ 57% of participants had serum 25(OH)D concentrations $\leq$ 40 nmol/L (Flicker et al., 2005).

The vitamin D form investigated was calcidiol in one study (Peacock et al., 2000), vitamin D_2 in two studies and vitamin D_3 in the remaining five studies. The highest vitamin D_2 or D_3 dose administered ranged from 20 to 250 μg/day (Burt et al., 2019; Bischoff-Ferrari et al., 2020), and in the one RCT administering calcidiol, the dose was 15 μg/day. In one RCT, the results from groups treated with 50 and 100 μg/day of vitamin D_3 were combined (Hin et al., 2016) and in another the dosage of vitamin D supplementation was lowered from 36 μg/day (= 250 μg/week) to 25 μg/day due to the discontinuation of the weekly dose preparation (Flicker et al., 2005). Four RCTs used calcium as a co-intervention, with the specific calcium supplementation doses ranging between 600 and 1,000 mg/day. Of these, one RCT supplied participants with enough calcium (maximum 600 mg/day) to reach the recommended daily amount of 1,200 mg, if they were not already getting that amount through their diet (Burt et al., 2019). One RCT with 8 intervention arms had used a combination of co-interventions with omega-3 fatty acids 1 g/day and either a control exercise program or a strength exercise program (Bischoff-Ferrari et al., 2020) (see evidence table in Appendix C.3). The duration of intervention across studies ranged from one to five years (Grant et al., 2005).

The studies reported two different types of fracture outcomes: (i) the number of participants who sustained at least one fracture (i.e., risk of fracture) and/or (ii) the number of fractures (i.e., fracture counts). The number of participants who sustained at least one fracture was of primary interest for this assessment and was reported in six RCTs, which are plotted in Figure 13. Two RCTs reported only on the number of fractures (Peacock et al., 2000; Bischoff-Ferrari et al., 2020). Burt et al. (2019) reported on both types of outcomes. Data on the number of fractures were considered as complementary evidence.

The available RCTs were very heterogeneous regarding how bone fractures were assessed and reported. Endpoints included low-trauma fractures, vertebral fractures, and any fracture, whereas in half of the RCTs bone fractures were not further specified. Some studies only reported fractures as an adverse event, while others included them as a primary or tertiary outcome (Appendix C.3). Five RCTs had reported to confirm fractures by x-ray reports or medical records when available, while the other three fractures were self-reported (Prince et al., 2008; Macdonald et al., 2013; Hin et al., 2016). This heterogeneity in fracture types and reporting methods precluded a quantitative synthesis of the evidence.

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Vitamin D_2 or D_3 supplementation at doses between 20 and 250 μg/day did not appear to significantly increase the risk of having a bone fracture when compared to control doses (Figure 13). Only one RCT reported a (non-significantly) higher risk of fractures with vitamin D supplementation versus placebo (Hin et al., 2016). In that RCT, 305 community-dwelling males and females aged 65 years with mean baseline serum 25(OH)D concentrations $>$ 50 nmol/L were randomised to consume vitamin D_3 at 50 or 100 μg/day or placebo for one year. Fractures of any type at any bone site were self-reported at 6 and 12 months of the study through an interview as adverse events. The number of participants sustaining at least one fracture by intervention arm is not reported. The number of subjects reporting at least one fracture at 12 months was 6/204 in the two vitamin D_3 arms combined and 1/101 in the placebo group. The difference was not statistically significant (p = 0.31). The study was at moderate RoB (tier 2), critical domains being outcome assessment (self-reported; not confirmed by x-ray or medical records) and blinding.

The number of bone fractures was not higher with vitamin D_3 at doses of 50, 100, and 250 μg/day than with control doses (Burt et al., 2019; Bischoff-Ferrari et al., 2020) (see Appendix C.3). In a 3-year multi-country study using a 3 × 2 factorial design, the incidence of non-vertebral bone fractures did not differ between groups treated with 0 and 50 μg/day of vitamin D_3 (IRR 1.03, 99%CI: 0.75, 1.43) (Bischoff-Ferrari et al., 2020). Similarly, in a 3-year study involving Canadian participants (Burt et al., 2019), the number of low-trauma fractures did not differ between treatment groups receiving 10, 100, and 250 μg/day of vitamin D_3 (fracture counts per group: 4/109, 3/100 and 5/102, respectively). Likewise, a 4-year study in US participants, aged 60 years or older found no significant difference in the number of bone fractures in individuals receiving 15 μg/day of calcidiol (33 events; n = 132) compared to placebo (23 events; n = 135) (p = 0.680) (Peacock et al., 2000).

The heatmap for the RoB assessment is in Appendix B.4. The RoB was considered low (tier 1) in four RCTs and moderate (tier 2) in the other four. Critical domains were exposure characterisation (n = 2), outcome assessment (n = 2), and attrition (n = 4).

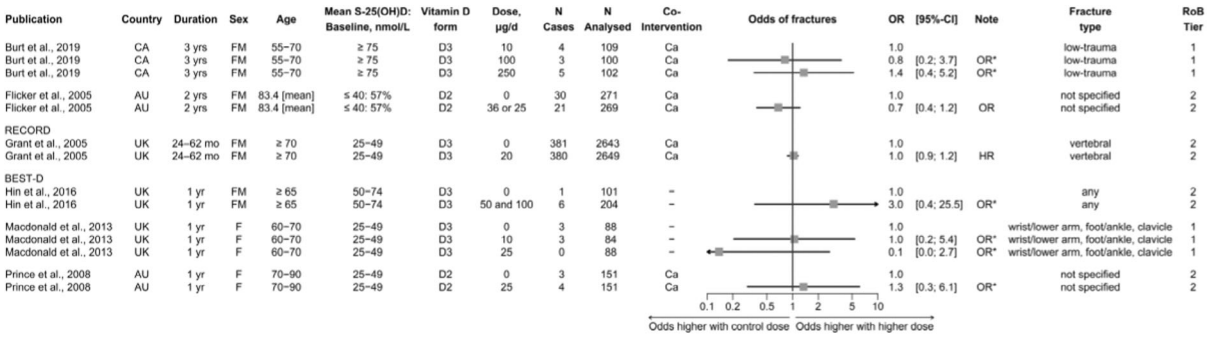

Figure 13: The effect of vitamin D_2 or vitamin D_3 supplementation on the odds of sustaining at least one fracture

For age, recruitment target range is presented, unless otherwise stated. Mean baseline S-25(OH)D concentrations were classified as follows, unless otherwise stated: $<$ 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, $\geq$ 75 nmol/L. 'Cases' indicates the number of participants who sustained at least one fracture during the intervention period. 'Note' indicates the effect size included in the forest plot. Pre-calculated effect sizes were used if available without performing conversions; please note that effect sizes can be therefore different from the one expressed in the plot title.

Abbreviations: AU, Australia; BEST-D: Biochemical Efficacy and Safety Trial of vitamin D; CA, Canada; Ca, calcium; CI, confidence interval; F, females; HR: hazard ratio; M, males; OR, odds ratio; RECORD: Randomised Evaluation of Calcium Or vitamin D; RoB: risk of bias; S-25(OH)D; serum 25-hydroxyvitamin D; UK, United Kingdom. **Note**: Burt et al. (2019) provided calcium supplementation to participants with dietary intake of less than 1,200 mg per day. Flicker et al. (2005) started with a vitamin D dose of 36 μg/day [= 250 μg/week], but due to the discontinuation of the preparation of commercial 250 μg-tablets during the intervention, they switched to 25 μg-tablets. Hin et al. (2016) combined the results of the groups treated with 50 μg and 100 μg of vitamin D_3. *Effect size was computed based on the reported case numbers because pre-calculated effect sizes were not available.

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The Panel considers that the limited evidence available does not suggest a positive relationship between the intake of vitamin D supplements at doses between 20 and 250 μg/day consumed for 1–5 years and the risk of having a bone fracture or the number of bone fractures in adults (≥ 55 years) and post-menopausal women. The Panel notes that, except for one RCT (Burt et al., 2019), all doses investigated were at or below the current UL, and that, except for Grant et al. (2005), these studies have not been designed to assess bone fractures. The Panel also notes the heterogeneity in the type and site of fractures reported.

3.5.2.2. Falls (sQ4b)

Out of the eight RCTs that reported on bone fractures, seven also reported on falls. Four additional RCTs that investigated the effect of vitamin D supplementation on the risk of falls only for a period of at least 1 year were included (Uusi-Rasi et al., 2015; Smith et al., 2017; LeBoff et al., 2020a; Appel et al., 2021), bringing the total number of RCTs for this clinical endpoint to 11.

Five RCTs were conducted in Europe, one in Canada, two in Australia and three in the USA (Figure 14 and Appendix C.3). The RCTs were conducted in adults and post-menopausal women, mostly Caucasian, with an average age between 64 and 84 years, who were living in the community. Only one RCT included residents in nursing homes or hostels (Flicker et al., 2005). Four RCTs included females only and the other seven RCTs included both sexes. Four RCTs had recruited participants with an elevated fall risk or history of falls in the previous year (Prince et al., 2008; Uusi-Rasi et al., 2015; Appel et al., 2021; Bischoff-Ferrari et al., 2022).

Mean baseline serum 25(OH)D concentrations were between 25 and 49 nmol/L in four RCTs, between 50 and 74 nmol/L in four RCTs and ≥ 75 nmol/L in two RCTs, while one RCT reported ~ 57% of participants had concentrations ≤ 40 nmol/L (Flicker et al., 2005). The vitamin D form investigated was vitamin D_2 in two studies and vitamin D_3 in the remaining studies, with the highest dose administered varying widely between 20 and 250 μg/day (Burt et al., 2019; Bischoff-Ferrari et al., 2022). Five RCTs used calcium as a co-intervention with doses ranging between 600 and 1,000 mg/day, or as needed to approximate total daily intake to between 1,200–1,400 mg/day. Four RCTs employed a factorial design, incorporating various treatment components in addition to vitamin D_3. These included calcium (Grant et al., 2005), an exercise program (Uusi-Rasi et al., 2015), n-3 fatty acids (LeBoff et al., 2020a) or a combination of n-3 fatty acids and exercise program (Bischoff-Ferrari et al., 2022) (see evidence table in Appendix C.3). The duration of intervention across studies ranged from one year and up to approximatively five years (Grant et al., 2005; LeBoff et al., 2020a).

One RCT used a response-adaptive randomization method that included a dose-finding stage (for fall prevention) followed by a best-dose stage (Appel et al., 2021). However, this approach made the overall dosing inconsistent and difficult to evaluate. Therefore, only the results from the dose-finding stage were considered in this assessment.

The 11 eligible RCTs primarily reported on two types of fall outcomes: (i) the number of participants who fell at least once (i.e., risk of falling) and (ii) the number of falls (i.e., fall counts). The primary focus of the review was on the number of fallers, which all RCTs reported on. Of these, 10 RCTs reported on the number of participants that fell at least once, one RCT on the number of participants that fell at least twice (LeBoff et al., 2020a) and one on both (Prince et al., 2008). Five RCTs reported on the number of falls and were used as complementary evidence. Some RCTs also recorded injurious falls or falls requiring hospitalisation. These endpoints are not discussed below.

Vitamin D_2 or D_3 at doses between 20 and 80 μg/day did not increase the risk of falling at least once or at least twice when compared to control doses or placebo in older adults (Figure 14 and Appendix C.3), either at mean baseline serum 25(OH)D concentrations < 50 nmol/L (Flicker et al., 2005; Grant et al., 2005; Prince et al., 2008; Smith et al., 2017) or > 50 nmol/L (Uusi-Rasi et al., 2015; LeBoff et al., 2020a).

At doses of vitamin D_3 ≥ 100 μg/day, three RCTs (Smith et al., 2017; Burt et al., 2019; Appel et al., 2021) reported a marginally (not statistically significant) higher risk of falling at least once at high vitamin D doses versus control doses or placebo. In the RCT by Burt et al. (2019), which provided vitamin D_3 at doses 10, 100 or 250 μg/day for 3 years, the highest risk of falling at least once was observed in the 100 μg/day dose, suggesting that the effect may not be specifically attributed to the vitamin D dose administered. In the second RCT (Appel et al., 2021), supplementation with vitamin D_3 alone at doses of 5, 25, 50 or 100 μg/day was provided for 2.5 years to older adults (≥ 70 years) with mean serum 25(OH)D > 50 nmol/L. The 50 and 100 μg/day groups had a (non-statistically significant) higher risk of falling at least once as compared the 5 μg/day, and the risk did not significantly differ across groups (Figure 14).

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In the 1-year RCT (Smith et al., 2017) 163 Caucasian overweight or obese postmenopausal women with mean serum 25(OHD) > 50 nmol/L and a relatively high history of falls in the previous year (~ 32%) were randomised to consume vitamin D_3 supplements at doses of 0, 10, 20, 40, 60, 80, 100 or 120 μg/day together with calcium to reach 1,200 to 1,400 mg/day. Since the primary outcomes of the study were serum 25(OH)D and PTH concentrations (see Gallagher et al. (2012) for the study design) and the study may have been underpowered for falls, the authors decided to group doses into clusters for data analysis based on the observation (visual inspection) that groups receiving 40, 60 or 80 μg/day had lower faller rates (i.e., subjects with at least one fall at the end of trial), and this group was taken as the reference. In multivariate logistic regression adjusting for age, BMI, alcohol use, smoking status and history of falls in the 12 months prior to the study, OR (95% CI) for placebo, 10–20 μg/day and 100–120 μg/day groups versus the 40–60-80 μg/day group were 3.86 (1.24–12.04; p_{adj} = 0.063), 3.15 (1.24–7.99; p_{adj} = 0.063), and 5.63 (2.14–14.85; p_{adj} = 0.0027), respectively, with no significant differences between the other groups (placebo vs. 10–20 μg/day, placebo vs. 100–120 μg/day). Owing to the low number of participants per intervention arm, it is unclear whether adjustment for variables which could have an impact on faller rates can fully account for the possibility of a failure in randomization regarding this endpoint. Figure 14 shows crude ORs taking the placebo group as reference. This study had a moderate RoB (tier 2), critical domains being outcome assessment (falls were retrospectively self-reported), attrition (analysis likely conducted in completers) and other sources of bias (comparability of dose groups at baseline unclear; dose grouping and data analysis were data-driven and not pre-planned). The Panel notes that this study does not show an increased risk of falling at vitamin D intakes of 100–120 μg/day versus placebo or intakes close to the adequate intake (10–20 μg/day).

In relation to the number of falls, multiple studies have shown that vitamin D_2 or D_3 at doses between 20 and 50 μg/day, and up to 250 μg/day in one RCT, did not significantly increase the number of falls when compared to control doses (Appendix C.3) (Flicker et al., 2005; Wood et al., 2014; Uusi-Rasi et al., 2015; Smith et al., 2017; Burt et al., 2019; Bischoff-Ferrari et al., 2022). In fact, most studies have found the effect in the dose range of 20 and 50 μg/day being either protective or null. However, Burt et al. (2019) reported a higher number of falls with 100 μg/day, but not with 250 μg/day, of vitamin D_3 when compared to a control dose (fall count 11/100, 6/102 and 4/109, respectively), suggesting that the observed effect is not specifically related to the dose of vitamin D. A higher number of falls was reported in the 20 μg/day arm when compared to placebo over a study period of 1 year in women with a history of falls in the previous year (rate of falls = 132.1 vs. 118.2 per 100 persons per year; IRR, 1.08 95% CI: 0.78, 1.52) (Uusi-Rasi et al., 2015). The same study reports a (non-significant) protective effect when investigating the risk of falling at least once (Figure 14).

No further analysis was performed on the available data.

The RoB was considered to be low (tier 1) for one RCT (Uusi-Rasi et al., 2015) and moderate (tier 2) for the remaining 10 RCTs. Critical domains were exposure characterisation (n = 5), outcome assessment (n = 5), and attrition (n = 4) (see heatmap in Appendix B.5).

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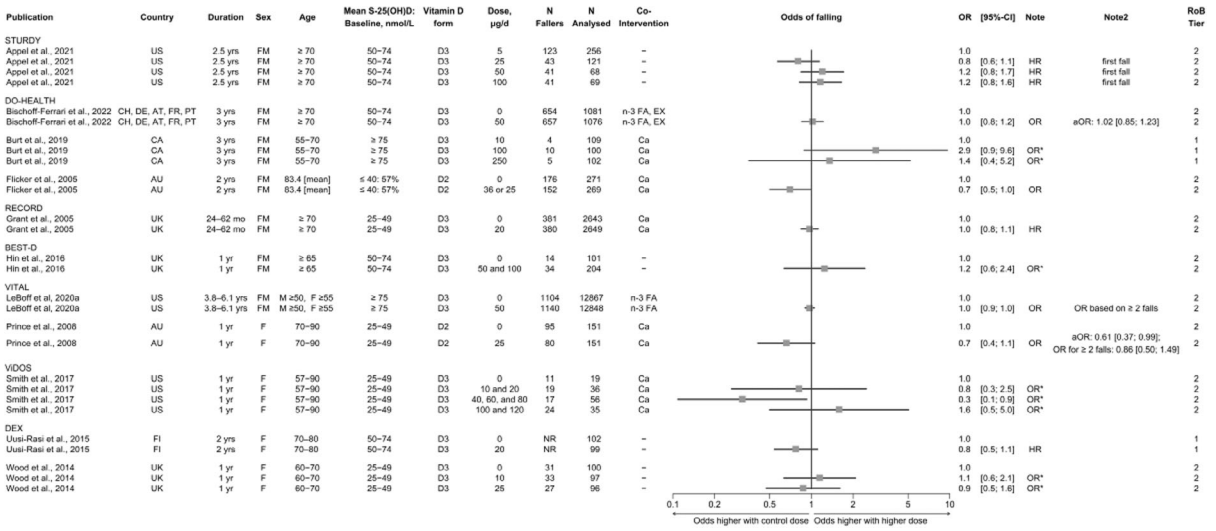

Figure 14: The effect of vitamin D supplements on the odds of falling at least once or twice.

For age, recruitment target range is presented, unless otherwise stated. Mean baseline S-25(OH)D concentrations were classified as follows, unless otherwise stated: < 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, ≥ 75 nmol/L. 'Fallers' indicate the number of participants who encountered one fracture or more. 'Note' indicates the effect size included in the forest plot. Pre-calculated effect sizes were used if available without performing conversions; please note that effect sizes can be therefore different from the one expressed in the plot title. 'Note2' indicates the adjusted effect size, if reported in the publication, or further information on the outcome. **Abbreviations:** AT, Austria; AU, Australia; aOR, adjusted odds ratio; BEST-D: Biochemical Efficacy and Safety Trial of vitamin D; CA, Canada; CH, Switzerland; CI, confidence interval; DE, Germany; DEX; Vitamin D and Exercise in Falls Prevention; DO-HEALTH: Vitamin D_3 - Omega3 - Home Exercise - Healthy Aging and Longevity Trial; EX, exercise program; F, females; FA, fatty acids; FI, Finland; FR, France; HR, hazard ratio; M, males; NR, not reported; OR, odds ratio; PT, Portugal; RECORD, Randomised Evaluation of Calcium Or vitamin D; RoB, risk of bias; S-25(OH)D, serum 25-hydroxyvitamin D; STURDY, Study to Understand Fall Reduction and Vitamin D in You; UK, United Kingdom; US, United States; ViDOS, Vitamin D supplementation in Older Women. **Note**: Flicker et al. (2005) started with 250 µg/week [= 36 µg/day], but due to the discontinuation of the preparation of commercial 250 µg-tablets during the intervention, they switched to 25 µg-tablets. Hin et al. (2016) and Smith et al. (2017) combined the results of the groups treated with higher vitamin D_3 doses. Uusi-Rasi et al. (2015) did not report raw data, i.e., the number of fallers. Bischoff-Ferrari et al. (2022) adjusted for study site, sex, age, previous fall, baseline BMI, and baseline use of walking aids. Prince et al. (2008) adjusted for baseline height as difference was observed between groups.

*Effect size was computed based on the reported case numbers because pre-calculated effect sizes were not available.

The Panel considers that the available BoE from RCTs does not suggest a positive relationship between vitamin D supplements at doses between 20 and 250 µg/day consumed for 1–5 years and the risk of falling at least once or the number of falls in adults > 55 years of age and post-menopausal women. The Panel notes the heterogeneity across studies on how falls were defined and assessed, that falls were rarely a primary outcome, that most studies investigated doses at or below the current UL, and that they were at moderate RoB (tier 2).

3.5.2.3. BMD/BMC and indices of bone strength (sQ4c)

Data from PCs and RCTs investigating vitamin D doses < 100 µg/day in adults and < 50 µg/day in children were not extracted because no adverse effects on BMD/BMC or related indices of bone strength were reported (Section 2.1.1.3). The results of the 14 RCTs and the 16 PCs excluded from data extraction for BMD/BMC (sQ4c) are briefly summarised below. For a list of these references see Appendix E.

Of the 14 RCTs, 11 tested supplemental doses of vitamin D ≤ 35.7 µg/day and only three investigated doses of vitamin D between 50 and 71.4 µg/day (Aloia et al., 2005; Larsen et al., 2017; LeBoff et al., 2020b). The intervention period ranged between 1 year (Andersen et al., 2008; Zhu et al., 2008a; Macdonald et al., 2013) and 5 years (Zhu et al., 2008b; Larsen et al., 2017). In all RCTs, the populations sampled were either adults and older adults (≥ 55 years of age) or post-menopausal women, except for

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Andersen et al. (2008) which included both children (aged 10–14 years) and adults. The vitamin D form investigated was calcidiol (15 μg/day) in one study (Peacock et al., 2000) and either vitamin D_2 or D_3 in the remaining studies. These 14 RCTs either showed a positive (Dawson-Hughes et al., 1995; Zhu et al., 2008b; Macdonald et al., 2013; Uusi-Rasi et al., 2015; Larsen et al., 2017) or a null effect on BMD, but no studies demonstrated a negative effect of vitamin D on BMD at the doses tested.

A total of 16 PCs were identified which investigated the relationship between serum 25(OH)D concentrations and BMD, BMC, and/or bone strength. Of these, six studies were in children and adolescents (Lehtonen-Veromaa et al., 2002; Breen et al., 2011; Sayers et al., 2012; Hauksson et al., 2016; Zhu et al., 2017; Yang et al., 2019). Follow-up periods ranged from 2 to 17 years (Yang et al., 2019) and the mean baseline serum 25(OH)D concentrations from 34 to 88 nmol/L (Breen et al., 2011). None of the PCs suggested an association between higher serum 25(OH)D concentrations and adverse effects on measures of bone health. On the contrary, some studies suggested positive associations between higher serum 25(OH)D concentrations and these endpoints (del Puente et al., 2002; Lehtonen-Veromaa et al., 2002; Ensrud et al., 2009; Breen et al., 2011; Sayers et al., 2012; Steingrimsdottir et al., 2014; Swanson et al., 2015; Zhu et al., 2017; Yang et al., 2019; Bevilacqua et al., 2021; Thompson et al., 2021). For a detailed description of these studies see section 3.3.3.4 of the technical report (Lamberg-Allardt et al., 2023).

A total of 6 RCTs (reported in 7 publications) investigating vitamin D supplementation doses ≥ 100 μg/day in adults and ≥ 50 μg/day in children were eligible for data extraction. Some of these studies had multiple doses of vitamin D (including doses < 50 and < 100 μg/day in adults and children, respectively), and within-study dose–responses are also considered in the assessment below. Of the six RCTs, five RCTs were conducted in adult populations and one in children and adolescents. Four of the five RCTs in adults had also reported on fractures and falls and were reviewed in earlier sections. The evidence tables are available in Appendix C.4.

Children and adolescents

A RCT in children and adolescents was conducted in Lebanon (El-Hajj Fuleihan et al., 2006; Al-Shaar et al., 2013) with a sample of healthy participants aged 10–17 years. Mean baseline 25(OH)D concentrations were 30 and 39 nmol/L for females and males, respectively, and participants had normal baseline serum calcium levels and body weight for age. Approximatively 20% of female participants were premenarcheal at study entry. Participants were divided into three groups receiving either placebo or vitamin D_3 at weekly doses of 35 μg or 350 μg, i.e., equivalent of 5 and 50 μg/day, respectively, for one year. BMD and BMC, as well as hip structural parameters such as cross-sectional area, outer diameter, section modulus, and buckling ratio, were assessed by DXA (Appendix C.4). Results for males and females were analysed separately.

There was no evidence for an adverse effect of vitamin D supplementation on any of the endpoints assessed in females or males, but rather a significant beneficial effect was observed for females (Appendix C.4) (El-Hajj Fuleihan et al., 2006; Al-Shaar et al., 2013). The RoB, according to sex, was considered low (Tier 1) for females and moderate (Tier 2) for males, with exposure characterisation and selective reporting being critical domains for males (heatmap in Appendix B.6).

The Panel notes that the only RCT available in children at doses up to 50 μg/day does not suggest a positive relationship between the intake of vitamin D supplements and adverse effects on BMD or BMC.

Adults

Of the five RCTs among adults, two were conducted in Norway (Jorde et al., 2010; Grimnes et al., 2012), one in Canada (Burt et al., 2019), one in USA (Smith et al., 2018) and one in Lebanon (Rahme et al., 2017). Most participants were Caucasians. Two RCTs included females only while the rest included both sexes. The populations recruited were generally older, with the mean age greater than sixty years in four RCTs, and 47 years in one RCT that recruited participants aged 21 to 71 years (Jorde et al., 2010). In one RCT participants had normal weight (Grimnes et al., 2012) and were either overweight or obese in the other four RCTs.

Mean baseline 25(OH)D concentrations were greater than 70 nmol/L in two RCTs, between 50 to 62 nmol/L in two other RCTs and below 50 nmol/L in one RCT. Vitamin D_3 was the only form of vitamin D investigated and the highest dose investigated ranged from 94 to 250 μg/day. While the labelled vitamin D dose in Rahme et al. (2017) was 84 μg, the analysed dose was in fact 94 μg. All RCTs used calcium as a co-intervention with doses ranging between 500 and 1,000 mg/day, or as needed to approximate a total daily intake between 1,200–1,400 mg/day (Smith et al., 2018; Burt

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et al., 2019). The duration of the intervention was one year in four RCTs and three years in Burt et al. (2019).

All RCTs in adults reported on areal BMD assessed by DXA at different sites: lumbar spine (n = 4), total hip (n = 4), and femoral neck (n = 3). The outcome variables differed across studies (end-of-study values, percent change from baseline, absolute change from baseline), which precluded a quantitative synthesis of the BoE. Data on total body BMD was extracted (see Appendix C.4) but not plotted or discussed below because of not being informative about osteoporotic fracture risk in older adults, including postmenopausal women. One RCT (Burt et al., 2019) also measured site-specific volumetric BMD at the tibia and radius and bone strength (failure load) using high-resolution peripheral quantitative computed tomography (HR-pQCT).

No adverse effect of vitamin D_3 supplementation at doses between 94 to 250 μg/day was observed on areal BMD assessed by DXA at the lumbar spine, femoral neck, or total hip, as compared to lower control doses or placebo. Areal BMD at these sites was unchanged or even slightly improved, with no significant differences between groups (Figure 15).

In the only RCT that assessed volumetric BMD using HR-pQCT (Burt et al., 2019), 311 males and females aged 55–70 years who were overweight on average and had serum 25(OH)D concentrations at baseline >75 nmol/L consumed vitamin D_3 supplements at doses of 10, 100 or 250 μg/day with calcium (to reach 1,200 mg/day) for 3 years. End of trial radial and tibial total volumetric BMDs were significantly lower in the 250 μg/day and 100 μg/day (only radial) groups than in the control group. Changes from baseline were − 3.5%, −2.4% and − 1.2% for total volumetric BMD at the radius and − 1.7%, −1% and − 0.4% for volumetric BMD at the tibia in the 250, 100 and 10 μg/day treatment arms, respectively. A significant negative dose–response relationship between vitamin D_3 and total volumetric BMD was found at both bone sites ($p < 0.001$ for group × time interaction). Bone strength (failure load) also decreased over the duration of the trial in all groups, with no significant differences among them at either site (Appendix C.4).

End of trial cortical and trabecular volumetric BMD, changes in the respective variables over the duration of the trial, cortical porosity, and trabecular number at both bone sites (tibia and radius) were secondary outcomes of the study. Results for cortical volumetric BMD at the tibia and radius were similar to those reported for total volumetric BMD at both sites. Trabecular volumetric BMD increased over trial duration, with lower gains for the 250 μg/day group at the radius only. Group x time interactions were not significant for the remaining endpoints and group comparisons.

The Panel notes that the only RCT identified reporting on volumetric BMD at the radius and tibia showed negative dose–response relationships with vitamin D supplementation at doses of 10, 100 and 250 μg/day over three years in participants with a mean baseline serum 25(OH)D > 75 nmol/L. These findings did not translate into significant differences in bone strength (failure load) among groups at the radius or the tibia, or in an increased risk of falls or low-trauma fractures (see Section 3.5.2.1). The Panel however notes that the study was not designed to assess falls or fractures.

All five RCTs were considered to be at low RoB (tier 1). See heatmap in Appendix B.6.

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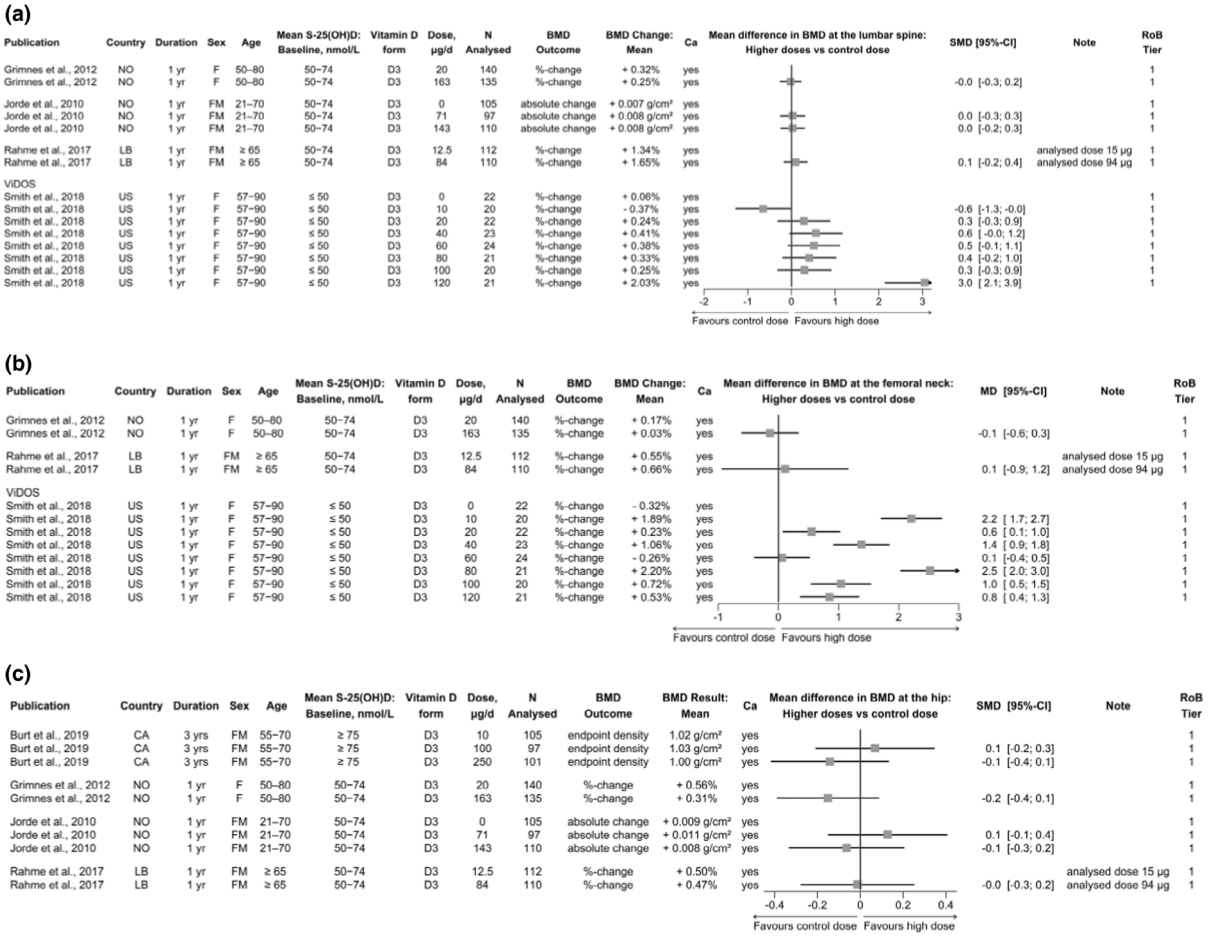

Figure 15: The effect of high doses of vitamin D_3 supplements on the areal BMD at the (a) lumbar spine, (b) femoral neck and (c) total hip among general adult populations

For age, recruitment target range is presented. Mean baseline S-25(OH)D concentrations were classified as follows, unless otherwise stated: < 25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, ≥ 75 nmol/L. 'BMD Result/Change' indicates the results/changes reported in the publications. 'Ca' indicates whether calcium was provided as a co-intervention. Effect size was standardised mean difference because the outcome metrics were different, except for plot (b) where the effect size was standardised mean difference because all studies reported %-changes from baseline. **Abbreviations**: BMD, bone mineral density; CA, Canada; CI, confidence interval; F, females; M, males; MD, mean difference; NO, Norway; LB, Lebanon; RoB: risk of bias; SMD, standardised mean difference; S-25 (OH)D; serum 25-hydroxyvitamin D; US, United States; ViDOS; Vitamin D supplementation in Older Women. **Note**: In Rahme et al. (2017), the analysed test dose was close to 100 µg/day, which is why it was decided to include the study in the evidence synthesis.

The Panel considers that the available evidence does not suggest a positive relationship between the intake of vitamin D supplements (in co-supplementation with calcium) at doses of up to 250 µg/day consumed for 1 and up to 3 years and adverse effects on areal BMD at different bone sites assessed by DXA.

The Panel notes the negative dose–response relationship reported between vitamin D supplementation at doses of 10, 100 and 250 µg/day and volumetric BMD at the radius and tibia in one RCT (Burt et al., 2019), which did not translate into differences in measures of bone strength, risk of falls or risk of bone fractures, although the study was not primarily designed for falls or fractures. The Panel considers that the clinical relevance of these findings is unclear.

3.5.2.4. Mechanisms of toxicity

There is some evidence that high vitamin D supplementation with intermittent high bolus doses given annually or monthly increases the risk of falls and fractures. In a RCT (Sanders et al., 2010) in 2,256 community-dwelling vitamin D replete females at high risk of fractures aged 70 years or older,

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12,500 μg of vitamin D_3 given annually for 3–5 years significantly increased the risk of falls (IRR 1.15, 95% CI 1.02, 1.30) and of fractures (IRR 1.26, 95% CI 1.00, 1.59) versus placebo (15% more falls and 26% more fractures). The increased risk of fractures was observed for fractures associated with falls and fractures not associated with a fall. In another RCT (Bischoff-Ferrari et al., 2016) in 200 men and women aged >70 years with a previous fall, monthly supplementation with vitamin D_3 (1,500 μg) or vitamin D_3 (600 μg) plus calcidiol (300 μg) for one year significantly increased the incidence of falls and number of falls as compared to monthly supplementation with lower doses of vitamin D_3 alone (600 μg). No differences were detected between low and higher vitamin D monthly supplementation in serum calcium or creatinine levels, urinary calcium excretion or PTH inhibition in the 12 months of the study.

It has been suggested by the authors that subjects receiving high-dose monthly vitamin D may have increased physical activity and thus a higher chance of falling (Bischoff-Ferrari et al., 2016), or that very high and subsequently very low levels of serum 25(OH)D resulting from annual doses may be causal (Sanders et al., 2010). However, the Panel notes that there are no known mechanisms for the increased risk of falls and fractures associated with high-dose intermittent vitamin D supplementation.

Burt et al. (2019) propose that, if the observed vitamin D dose-dependent loss of volumetric BMD observed in the trial represents a real effect, it might be related to the observed combination of an increased plasma marker of bone resorption, c-telopeptide of type 1 collagen (CTx), and suppression of PTH seen in the 250 μg/day vitamin D_3 group, owing that high-dose vitamin D with extra calcium supplementation appears to be associated with increased levels of $1,25(OH)_2D$, which stimulates osteoclastogenesis and differentiation (Aloia et al., 2015). However, calcium supplements were provided in the trial to reach a combined intake from diet and supplements of 1,200 mg/day and serum levels of $1,25(OH)_2D$ were not measured. It is also suggested that high-dose vitamin D may also suppress PTH by direct action on parathyroid cells or indirectly by enhancing intestinal calcium absorption, which may reduce bone formation and, in combination with a direct effect of high vitamin D on osteoclast activity (as supported by increased CTx), could result in the dose-related accelerated decline in observed volumetric BMD. The authors proposed that the increase in trabecular and decrease in cortical volumetric BMD could reflect this increased resorption, compatible with tabularization of cortical bone. The Panel notes, however, that serum PTH and CTx were within the normal range during the study.

The Panel considers that there are no well-established mechanisms by which daily or weekly doses of vitamin D up to 250 μg/day could have a detrimental effect on muscle function or bone health.

3.5.2.5. Evidence integration and uncertainty analysis

Evidence from RCTs on the effects of vitamin D supplementation lasting ≥ 1 year on falls and bone fractures is limited. Falls and fractures were rarely a primary outcome, so that RCTs were not designed to assess these endpoints. RCTs were also very heterogeneous regarding the definition, assessment, and reporting of fall- and fracture-related endpoints, which precluded a quantitative synthesis of the evidence. In addition, most studies investigated vitamin D doses ≤ 100 μg/day, the current UL for adults (Sections 3.5.2.1 and 3.5.2.2), and were mostly at moderate RoB, critical domains being exposure characterisation, outcome assessment and attrition. These RCTs do not suggest an increased risk of falls or fractures at vitamin D doses up to 250 μg/day consumed for 1 and up to 5 years versus control doses or placebo.

A wider BoE is available in relation to total body and site-specific BMC/BMD assessed by DXA. Evidence from 14 RCTs using doses of vitamin D below the current UL for adults (100 μg/day; n = 13) and children (50 μg/day; n = 1) for the respective population groups did not report adverse effects on markers of bone health. This is supported by evidence from 16 PCs which investigated the relationship between baseline serum 25(OH)D (which ranged from 34 to 88 nmol/L) and these endpoints in adults (n = 10) or children (n = 6) (Section 3.5.2.3).

The five RCTs conducted in adults which provided vitamin D in co-supplementation with calcium at doses at the current UL and up to 250 μg/day for 1 and up to 3 years did not show adverse effects on areal BMD at different bone sites (lumbar spine, femoral neck, total hip) assessed by DXA. These studies were at low RoB for this endpoint. Results from the only RCT in children giving 50 μg/day vitamin D are consistent with this conclusion.

The only RCT in older adults that assessed volumetric BMD and bone strength (failure load) at the radius and tibia using HR-pQCT was also the one testing the highest doses of vitamin D (up to 250 μg/day) for the longest period (3 years) (Burt et al., 2019). The negative dose–response relationship reported between vitamin D supplementation at doses of 10, 100 and 250 μg/day and volumetric BMD

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was unexpected and did not translate into differences in measures of bone strength. In addition, there are no convincing mechanisms that could explain such effect, and the clinical relevance of the findings is unclear. For these reasons, the Panel considers that, as proposed by the authors, the results of this RCT cannot be taken as a strong indication of skeletal harm associated to long-term vitamin D supplementation at doses of 250 μg/day in combination with calcium, and thus cannot be the basis to set a UL for vitamin D. The Panel notes, however, that potential adverse effects of high doses of vitamin D on markers of bone health should be nevertheless considered when establishing a UL for vitamin D.

3.5.2.6. Conclusions on musculoskeletal health

Based on the available evidence, the Panel concludes that:

- RCTs with vitamin D doses up to 250 μg/day consumed for 1 and up to 5 years do not suggest an increased risk of falls or fractures versus control doses or placebo.
- PCs do not suggest a relationship between higher serum 25(OH)D concentrations and lower areal BMD as assessed by DXA.
- RCTs do not suggest an adverse effect of high vitamin D intakes up to 50 μg/day in children and up to 250 μg/day in adults for ≥ 1 year on areal BMD at the lumbar spine, femoral neck, or total hip (or BMC in children) assessed by DXA.
- The RCT testing the highest doses of vitamin D (up to 250 μg/day) for the longest period (3 years) in older adults (Burt et al., 2019) also reported a negative dose–response relationship between vitamin D supplementation and volumetric BMD at the radius and tibia using HR-pQCT, which did not translate into differences in measures of bone strength (failure load).
- Potential adverse effects of vitamin D supplementation at doses of 250 μg/day in co-supplementation with calcium on markers of bone health may need to be considered when establishing a UL for vitamin D.

3.5.3. Other adverse health effects

3.5.3.1. Kidney stones

About 80% of all kidney stones in Western countries are made of calcium oxalate and, to a lesser extent, calcium phosphate. Low urine volumes, low urinary concentrations of magnesium and citrate, disproportionately acidic or alkaline urine pH, and a persistent increase in urinary excretion of calcium, oxalate, and uric acid contribute to kidney stone formation (Letavernier and Daudon, 2018). High calcium, sodium and animal protein intakes have been associated with a higher risk of kidney stones by fostering hypercalciuria, one of the main determinants of calcium-dependent kidney stone formation (Siener, 2021). Whereas dietary calcium may decrease the risk of kidney stones by decreasing intestinal absorption (and urinary excretion) of oxalate, calcium supplements between meals would not have such a protective effect (Bargagli et al., 2021).

The role and contribution of vitamin D to kidney stone formation is a matter of debate. A series of systematic reviews and meta-analysis has investigated the effect of vitamin D supplementation, with or without calcium, on the risk of nephrolithiasis in RCTs, and the relationship between serum 25(OH)D concentrations and kidney stone formation in PCs. In two Cochrane systematic reviews of RCTs on vitamin D supplementation for the prevention of bone fractures (Avenell et al., 2014) and all-cause mortality (Bjelakovic et al., 2014), risk of kidney stones was assessed in 11 and 4 RCTs, respectively. In both cases, there was evidence for a statistically significant increase in the incidence of renal calculi or renal insufficiency with vitamin D supplementation. However, one study (Jackson et al., 2006) comparing 1,000 mg/day of calcium plus 10 μg/day vitamin D_3 versus placebo accounted for 96.4% and 99.1%, respectively, of the weight in the meta-analysis (EFSA NDA Panel, 2012a). The Panel notes that in a reanalysis of the results of this study considering only subjects complying with the study protocol (Wallace et al., 2011), neither total calcium intake nor the use of calcium supplements at baseline were associated with the risk of kidney stones, and that additional calcium (and vitamin D) supplementation did not increase the risk of self-reported kidney stones in subjects who complied with the study treatment over widely variable baseline calcium intakes from food and personal supplements (from < 400 to > 1,490 mg/day). The Panel also notes that any effects on nephrolithiasis could not be attributed to vitamin D alone, as calcium intake was not controlled for.

Two meta-analyses of RCTs investigating the effect of vitamin D (D_2 or D_3) supplementation, with or without calcium, on kidney stones that excluded RCTs not controlling for calcium intake in the

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placebo group have been published thereafter by the same authors. The first meta-analysis included trials lasting ≥ 24 weeks (Malihi et al., 2016). In the 9 RCTs identified (9,619 participants) which reported at least one case of kidney stones, of which 8 used vitamin D doses > 20 μg/day and 6 lasted ≥ 1 year, no increased risk of kidney stones was observed when the vitamin D and placebo arms were compared (RR: 0.66, 95% CI: 0.41, 1.09; p = 0.10). Sensitivity analysis considering calcium co-supplementation, serum 25(OH)D at baseline, vitamin D dose or duration of the intervention did not significantly change the results. The second meta-analysis included RCTs with doses of vitamin D_2 or D_3 ≥ 70 μg/day and lasting ≥ 1 year (Malihi et al., 2019). In the 5 RCTs (1,336 participants) that reported at least one case of kidney stones, the risk of kidney stones was not significantly higher in the vitamin D arms versus placebo (RR = 1.26; 95% CI = 0.35, 4.58; p = 0.72).

In a systematic review and meta-analysis of observational studies (case–control (CC) studies n = 30; PCs n = 2), differences in serum 25(OH)D and serum $1,25(OH)_2D$ concentrations between kidney stone formers, calcium stone formers, hypercalciuria stone formers and normocalciuria stone formers versus controls, and between hypercalciuria stone formers versus normocalciuria stone formers, were assessed (Hu et al., 2017). Stone formers (22 CC, 1 PC), calcium stone formers (14 CC, 1PC) and hypercalciuria stone patients (9 CC) had significantly higher serum concentrations of $1,25(OH)_2D$ than controls, whereas serum 25(OH)D concentrations were only significantly higher in hypercalciuria stone formers versus controls (3 CC) and versus normocalciuria stone formers (5 CC). The authors concluded that, whereas high concentrations of circulating $1,25(OH)_2D$ were associated to kidney stone formation, both circulating 25(OH)D and $1,25(OH)_2D$ are higher in kidney stone formers with hypercalciuria. The Panel notes the low number of studies available assessing serum 25(OH)D in stone formers and the limited ability of case–control studies to establish causality.

The Panel considers that the available evidence on the relationship between vitamin D intake and risk of kidney stone formation cannot be used for establishing a UL for vitamin D.

3.5.3.2. Cardiovascular disease

Although most intervention and observational studies have reported an inverse relationship between vitamin D intake or status and CVD risk, some case control (Lindén, 1974), cross-sectional (Melamed et al., 2008), and prospective cohort studies (Vieth, 1999; Wang et al., 2008; Ginde et al., 2009) have suggested a U or J relationship between serum 25(OH)D concentrations and CVD risk and mortality, raising the hypothesis that high vitamin D intakes could lead to higher CVD risk through vascular calcification (Zittermann and Prokop, 2014).

The effect of vitamin D supplementation on CVD incidence and/or mortality has been recently investigated in large RCTs, such as the Vitamin D Assessment (ViDA) Study (Scragg, 2020), VITamin D and OmegA-3 TriaL (VITAL) (Manson et al., 2020), the Finnish vitamin D Trial (FIND) (Virtanen et al., 2022) or the D-health trial (Neale et al., 2022). Vitamin D_3 was provided monthly at doses of 2,500 μg (~ 83 μg/day) or 1,500 μg (~ 50 μg/day) in the ViDA and D-health trials, respectively, or daily at doses of 50 μg/day (VITAL) or 40 and 80 μg/day (FIND) for 3.3 (ViDA) to about 5 years (VITAL, FIND, D-health). No effect of vitamin D supplementation on cardiovascular events or cardiovascular mortality was observed in any of these trials.

Several systematic reviews and meta-analysis of RCTs on vitamin D supplementation and risk factors for CVD, CVD incidence and mortality have been conducted in the last years.

In a systematic review of RCTs on vitamin D supplementation and (established or potential) risk factors for CVD including 81 trials (Mirhosseini et al., 2018), no adverse effects of vitamin D on blood pressure (39 trials), the blood lipid profile (38 trials), hs-CRP (28 trials), pulse-wave velocity (PWV, 11 trials) or the augmentation index (10 trials), were observed in the meta-analyses conducted for these endpoints, or in sensitivity analysis considering vitamin D dose or status, duration of the intervention, age or BMI, where PWV and the augmentation index are measures of arterial stiffness. The average supplementation dose was ~ 75 μg/day. Achieved serum 25(OH)D concentrations ≥ 100 nmol/L were observed in trials with vitamin D supplementation at doses between 100 μg and 300 μg/day.

An umbrella review of previous systematic reviews and meta-analyses of RCTs (Rejnmark et al., 2017) focused on non-skeletal outcomes reported in vitamin D supplementation trials, which were often reported as secondary outcomes. Seven systematic reviews including a total of 21 RCTs reporting on CVD outcomes were identified. A total of 16 RCTs investigated vitamin D_2 or D_3 and 5 RCTs investigated activated vitamin D analogues (calcitriol or ED-71). Baseline serum 25(OH)D concentration was < 50 nmol/l in 6 RCTs, > 50 nmol/L in 7 RCTs and not reported in 8. Only one RCT lasted < 1 year. All meta-analyses and individual RCTs reported null findings of vitamin D supplementation (either alone or with calcium co-supplementation) on CVD incidence or mortality. This

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is in line with the results of a previous umbrella review of systematic reviews and meta-analyses of observational studies (on serum 25(OH)D concentrations) and RCTs (on vitamin D supplementation) on a vast range of endpoints, including CVD disease incidence and mortality (Theodoratou et al., 2014).

A more recent systematic review (Pei et al., 2022) on vitamin D supplementation (with or without calcium co-supplementation) and CVD incidence and mortality included 18 RCTs (70,278 participants) lasting between 1 and 6 years which provided yearly, monthly, weekly, or daily doses of vitamin D corresponding to daily doses between 10 and 100 μg/day, and up to 2,500 μg/day in one study (Brohult et al., 1973). No differences between vitamin D and placebo groups were reported for CVD mortality (9 trials; 63,227 participants; 736 vs. 759 events), myocardial infarction (14 trials; 46,194 participants; 552 vs. 566 events) or stroke (12 trials; 46,093 participants; 437 vs. 413 events), or in sensitivity analyses considering the number of subjects, number of events, vitamin D dose, pattern of administration, serum 25(OH)D at baseline and duration of the intervention.

The Panel notes that the available evidence from RCTs does not suggest a negative effect of vitamin D supplementation at doses up to 100 μg/day for up to five years on CVD incidence or mortality, or a positive relationship between serum 25(OH)D concentrations and these endpoints in PCs.

The Panel considers that the available evidence on the relationship between vitamin D intake or status and CVD incidence or mortality cannot be used for establishing a UL for vitamin D.

3.5.3.3. Cancer

Observational studies and RCTs (mostly designed for skeletal outcomes) have suggested an inverse association between 25(OH)D concentrations or vitamin D intake and cancer risk/mortality. Some observational studies, however, have reported U-shaped relationships between serum 25(OH)D concentration and cancer mortality. In the Uppsala Longitudinal Study of Adult Men (Michaelsson et al., 2010), a 2-fold increase in all-cause and cancer mortality rates was observed among men (mean age 71 years at baseline) in the lowest 10% ($<$ 46 nmol/L) and the highest 5% ($>$ 98 nmol/L) of plasma 25(OH)D concentrations compared with intermediate concentrations. A pooled nested case–control study of participants from 8 cohorts within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (Stolzenberg-Solomon et al., 2010) investigated the relationship between circulating 25(OH)D concentrations pre-diagnosis and the development of pancreatic cancer. Compared with serum 25(OH)D concentrations 50.0–$<$ 75.0 nmol/L (reference), no significant associations were observed at serum 25(OH)D concentrations $<$ 50.0 nmol/L, but a 2-fold increased risk was observed at 25(OH)D levels $\geq$ 100 nmol/L.

The recent large ViDA, VITAL, FIND and D-health trials on vitamin D supplementation (see Section 3.5.3.2) also assessed cancer risk and cancer mortality. Among them, only the D-health trial conducted in Australia, in which monthly doses of 1,500 μg ($\sim$ 50 μg/day) vitamin D_3 or placebo were given for 5 years to adults 60 years of age and older (n = 3,943), reported an increased risk of cancer mortality in the vitamin D_3 group compared to placebo. HR was 1.5 (95% CI 0.96–1.39), and decreased to 1.24 (95% CI, 1.01–1.54) when cases identified in the first 2 years of the study were excluded (Neale et al., 2022).

Vitamin D_3 supplementation had no effect on the incidence of all cancer in the ViDA study (2,500 μg/month for a median duration of 3.3 years; n = 5,110; [Scragg et al., 2018]) or the incidence of invasive cancer in the FIND trial (40 or 80 μg/day; n = 2,495; [Virtanen et al., 2022]) compared to placebo. The VITAL trial, a placebo-controlled 2 $\times$ 2 factorial design providing daily vitamin D_3 (50 μg) and marine omega-3 fatty acids (1 g) for the primary prevention of invasive cancer and CVD (median treatment duration was 5.3 years), included 25,871 US men aged $\geq$ 50 years and women aged $\geq$ 55 years, of which 5,106 African Americans. Vitamin D_3 did not reduce significantly total invasive cancer incidence (primary endpoint; HR = 0.96,95% CI, 0.88–1.06) but reduced total cancer mortality (HR = 0.83, 95% CI, 0.67–1.02), especially in analyses that accounted for latency by excluding the first year (HR = 0.79 95% CI, 0.63–0.99) or the first 2 years (HR = 0.75 95% CI, 0.59–0.96) of follow-up (Manson et al., 2020). The cumulative incidence curves for cancer mortality began to diverge clearly at 4 years.

An additional large (n = 2,385) RCT of long duration (median follow-up of 2.9 years) providing high doses of vitamin D (100 μg) or placebo was identified (Chatterjee et al., 2021). The Vitamin D and type 2 diabetes cancer outcomes study (D2dCA) recruited overweight or obese participants free of diabetes or cancer for the previous 5 years (mean age 60 years at baseline; mean serum 25(OH)D = 70 nmol/L). No effect of vitamin D supplementation on cancer risk, prostate cancer risk, or colorectal adenomatous polyps on elective colonoscopy was observed.

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Umbrella reviews, systematic reviews and meta-analyses of interventions exploring the relationship between vitamin D intake or status and cancer risk have been published.

In a meta-analysis of RCTs (Bjelakovic et al., 2014), vitamin D supplementation significantly (RR = 0.88; 95% CI: 0.78–0.98) reduced cancer mortality (4 trials, 44,492 participants duration of the intervention 5–7 years;) and had no effect on cancer incidence reported as an adverse event (14 trials, 49,707 participants). Similar results were found in a meta-analysis (Keum et al., 2019) of 10 RCTs on cancer incidence (6,537 cases; 3–10 years of follow-up; 54–135 nmol/L of attained concentrations of 25(OH)D in the intervention group) and 5 RCTs on cancer mortality (1591 cancer deaths; 3–10 years of follow-up; 54–135 nmol/L of attained concentrations of 25(OH)D in the intervention group). No effect of vitamin D_3 supplementation on cancer incidence was found, even at serum 25(OH)D concentrations exceeding $>$ 100 nmol/L, whereas vitamin D_3 supplementation significantly reduced cancer mortality (RR = 0.87, 95% CI, 0.79–0.96). Finally, in a systematic review and meta-analysis of 12 RCTs (Zhang et al., 2022) which included the ViDAL, VITAL, FIND, D-health and D2dCA trials mentioned above (72,669 participants), no relationship between vitamin D supplementation and cancer risk was found. Vitamin D supplementation was rather associated with a reduction in lung cancer mortality (RR 0.63, 95% CI 0.45–0.90) in sensitivity analyses. The umbrella review of systematic reviews and meta-analyses of RCTs previously mentioned ([Rejnmark et al., 2017]; see Section 3.5.3.2) also reports no overall beneficial or adverse effects of vitamin D supplementation on cancer risk.

The Panel considers that the available evidence on the relationship between vitamin D intake or status and cancer risk and mortality cannot be used for establishing a UL for vitamin D.

3.5.3.4. All-cause mortality

Observational studies have mostly described an inverse linear association between vitamin D status and mortality (Hutchinson et al., 2010; Johansson et al., 2012; Zittermann et al., 2012; Durazo-Arvizu et al., 2017; Gaksch et al., 2017). However, U-shaped, or reverse-J-shaped risk curves, have also been described between serum 25(OH)D concentrations and all-cause mortality (Jia et al., 2007; Michaelsson et al., 2010; Durup et al., 2012), with the lowest mortality rates generally found at serum 25(OH)D concentrations between 50 and 75 nmol/L.

Several reasons have been proposed to explain the latter observations. One refers to the analytical methods used to measure 25(OH)D concentrations. The reverse J-shaped association between serum 25(OH)D concentration and all-cause mortality was first reported in a 9-year follow-up (1991–2000) analysis of the Third National Health and Nutrition Survey (NHANES III, 1988–1994) (Sempos et al., 2013). A second re-assessment of 25(OH)D levels using standardisation methodology developed by the VDSP, led to an inverse linear relationship between all-cause mortality and serum 25(OH)D $<$ 40 nmol/L, and to a null association for serum 25(OH)D between 40 and 120 nmol/L. (Durazo-Arvizu et al., 2017). A second explanation refers to reverse causality: individuals with very high serum 25(OH)D concentrations could be taking vitamin D supplements due to poor health (Grant, 2015; Grant et al., 2016). The third explanation advocates to the lower concentrations of the active vitamin D metabolite $1,25(OH)_2D$ associated with high serum 25(OH)D which could, by themselves, increase mortality (Zittermann and Prokop, 2014).

Most recent systematic reviews and meta-analyses of RCTs and observational studies, however, mostly report a lower risk of all-cause mortality with increasing vitamin D intakes/serum 25(OH)D concentrations.

Two partially overlapping systematic reviews and meta-analyses of RCTs identified 56 and 50 RCTs including 95,286 and 74 655 participants, respectively, and reached similar conclusions (Bjelakovic et al., 2014; Zhang et al., 2019). In the first systematic review (Bjelakovic et al., 2014), which mostly included trials in women older than 70 years, vitamin D supplementation decreased mortality risk, an effect that was statistically significant only for vitamin D_3 in relation to cancer mortality. Serum 25(OH)D concentrations were $<$ 50 nmol/L in 26 trials and $<$ 50 nmol/L in 19 trials of the 45 reporting on this variable. There was no indication for a higher risk of mortality with higher doses (100 μg/day) of vitamin D. In the second systematic review (Zhang et al., 2019), vitamin D supplementation (30 trials $<$ 50 μg/day, 16 trials $>$ 50 μg/day) was not associated with all-cause mortality, cardiovascular mortality or non-cancer, non-cardiovascular mortality, whereas vitamin D supplementation significantly reduced the risk of cancer death (RR = 0.85, 95% CI = 0.74–0.97). In subgroup analyses, all-cause mortality was significantly lower in trials with vitamin D_3 than in trials with vitamin D_2. Again, there was no indication of higher mortality risk with higher vitamin D doses.

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In a meta-analysis of 32 prospective (cohort and nested case–control) observational studies (Garland et al., 2014), the hazard ratio for all-cause mortality comparing the lowest (< 22.5 nmol/L) to the highest (> 75 nmol/L) category of serum 25(OH)D was 1.9 (95% CI = 1.6, 2.2). Serum 25(OH)D concentrations < 75 nmol/L were associated with higher all-cause mortality than concentrations > 75 nmol/L.

Two re-analyses of individual participant data from PCs have also been published. One (Schottker et al., 2014) included data from 26,018 participants aged 50–79 years from 8 PCs from Europe and the US, and serum 25(OH)D quintiles were defined with cohort and subgroup specific cut-off values. Analysis using all quintiles showed curvilinear, inverse dose–response relationships between serum 25 (OH)D and all-cause mortality, CVD mortality (both in subjects with or without a history of CVD at baseline) and cancer mortality (only in subjects with a history of cancer). Results were consistent across countries, sexes, age groups and season of the blood draw. The second (Gaksch et al., 2017) included 26,916 participants (median age 61.6 years, 58% females) with a median serum 25(OH)D concentration of 53.8 nmol/L from eight European prospective cohorts. Serum 25(OH)D concentrations were standardised according to the VDSP. An inverse relationship between serum 25(OH)D concentration and all-cause mortality and CVD mortality was observed, whereas no significant association was reported for cancer mortality. No increased risk of mortality was observed at serum 25 (OH)D concentrations up to 125 nmol/L, although the number of individuals with serum 25(OH)D concentrations higher than 125 nmol/L was small (n = 172).

Finally, an umbrella review (Liu et al., 2022) included 296 meta-analyses of observational studies with 111 unique outcomes, 139 meta-analyses of RCTs comprising 46 unique outcomes, and 73 Mendelian randomization studies with 43 unique outcomes. Among all the outcomes assessed, all-cause mortality was the only endpoint for which identical conclusions (effect and level of statistical significance/direction) were reached across the three study types, suggesting that serum 25(OH)D concentrations were inversely related to all-cause mortality.

The Panel considers that the available evidence on the relationship between vitamin D intake or status and all-cause mortality cannot be used for establishing a UL for vitamin D.

3.6. Hazard characterisation

3.6.1. Selection of the critical effect

The critical effect on which the UL for vitamin D was established in 2012 for all population groups except infants (EFSA NDA Panel, 2018) is persistent hypercalcaemia (EFSA NDA Panel, 2012b). The UL was set at 100 μg/day for adults based on a NOAEL of 250 μg/day and an UF of 2.5. The same UL was established for adolescents and for pregnant and lactating women. The UL for children up to 10 years was set at 50 μg/day owing to their smaller body size.

Whereas the hypercalcaemic-hypercalciuric syndrome is a well-established adverse effect of vitamin D toxicity, adverse effects of high (daily or weekly) vitamin D doses on musculoskeletal health have only been reported in one RCT (Burt et al., 2019). A negative dose–response relationship was observed between vitamin D supplementation at doses of 10, 100 and 250 μg/day and volumetric BMD at the radius and tibia. Volumetric BMD decreased significantly more in the 100 μg/day (only at the radius) and 250 μg/day (both sites) groups than in the 10 μg/day (control) group, whereas measures of bone strength (failure load) did not significantly differ across groups during the study at either bone site. In the same RCT, no adverse effects of vitamin D supplementation on areal BMD (total hip) assessed by DXA were found.

The Fracture Risk Assessment Tool (FRAX) (Kanis, 2008), used to predict 10-year fragility (or osteoporotic, low-trauma) fracture risk, relies on the measurement of areal BMD by DXA, in combination with clinical risk factors. Areal BMD is the gold standard proposed by WHO for measuring bone density. However, it provides information mainly on bone mineral mass and not on bone quality. It has been suggested that, since bone strength is determined not only by bone mass, but also by bone morphology and microarchitecture, HR-pQCT, which is able to measure cortical and trabecular bone density and microarchitecture at peripheral skeletal sites could better predict fracture risk, even independently of areal DXA BMD or even FRAX. In the largest prospective study published to date testing this hypothesis (7,254 individuals, 765 incident fractures over 4.6 years follow-up; [Samelson et al., 2019]), peripheral skeleton failure load had the greatest association with the risk of fracture. HR was 2.40 (95% CI 1.98–2.91) for the tibia and 2.13 (1.77–2.56) for the radius per 1 SD decrease, whereas HRs for other bone indices ranged from 1.12 (95% CI 1.03–1.23) per 1 SD increase in tibia cortical porosity to 1.58 (1.45–1.72) per 1 SD decrease in radius trabecular volumetric BMD. Failure

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load was the strongest predictor of fractures at the tibia and radius, independently of femoral neck areal BMD and FRAX. It is important to highlight that no significant differences among vitamin D supplementation groups on failure load were observed at either bone site in the study by Burt et al. (2019).

The Panel notes that, although potential adverse effects of high-dose vitamin D supplementation on HR-pCT markers of bone health should be further explored, together with the ability of such markers to predict fracture risk, volumetric BMD cannot be used alone as a critical endpoint on which to base the UL for vitamin D.

The Panel also notes that there is no consistent evidence for adverse effects of vitamin D supplementation/high vitamin D status on the other endpoints assessed, and that available data on the relationship between vitamin D intake or status and risk of kidney stones, CVD, cancer or cause-specific/all-cause mortality cannot be used for establishing a UL for vitamin D.

Based on the available evidence, the Panel decides to select persistent hypercalcaemia/hypercalciuria as the critical effect on which to base the UL for vitamin D.

3.6.2. Derivation of the UL

3.6.2.1. Adults

The current UL for vitamin D (EFSA NDA Panel, 2012b) of 100 μg/day derives from a NOAEL for hypercalcaemia of 250 μg/day based on two studies in which doses of 234–275 μg vitamin D_3/day were administered to 10–15 healthy men with minimal sun exposure for 8 weeks to about 5 months without reported hypercalcaemia (Barger-Lux et al., 1998; Heaney et al., 2003). An UF of 2.5 was applied to cover the range of variation in the sensitivity of the population to possible adverse effects of vitamin D over the long-term.

The Panel has now re-assessed the literature on the effect of vitamin D on hypercalcaemia. In this update, the study of shorter duration (Barger-Lux et al., 1998) was not considered eligible for inclusion due to the fact that serum calcium was measured only once after baseline in the vitamin D_3 arms. Two additional studies have been identified in which vitamin D_3 was given alone at doses of 250 μg/day for 5–6 months to healthy and obese males and females (age 18–68 years, sample size 8–20 subjects per arm) with no reported cases of persistent hypercalcaemia (Drincic et al., 2013; Shirvani et al., 2020).

The Panel notes, however, that:

a) hypercalciuria may precede hypercalcaemia, and none of the RCTs giving vitamin D alone at doses of 250 μg/day assessed urinary calcium;
b) in one RCT (Billington et al., 2020), vitamin D supplements at doses of 250 μg/day for 3 years in co-supplementation with calcium to reach adequate intakes for the study population (1,200 mg/day) increased three times the risk of persistent hypercalciuria in older adults of both sexes with mean baseline serum 25(OH)D concentration of $>$ 75 nmol/L;
c) an increased risk of persistent hypercalciuria of the same magnitude was also reported in another RCT using the same doses of vitamin D in co-supplementation with calcium for 1 year (Aloia et al., 2018), even when calcium supplements were either reduced or withdrawn.

The Panel considers that a LOAEL for vitamin D should be set at 250 μg/day based on findings of persistent hypercalciuria. The Panel notes that this is an early marker of adverse events, and that it is reversible with vitamin D and/or calcium supplements withdrawal.

The Panel notes that the uncertainties previously considered by the NDA Panel for applying an UF of 2.5 to derive the UL (i.e. young males only, small sample size, short duration, minimal sun exposure) are addressed in the RCTs by Aloia et al. (2018) and Billington et al. (2020) (i.e., both males and females with adequate vitamin D status, older age, larger sample size, longer duration). The Panel considers, however, that an UF of 2.5 is still appropriate to account for the absence of a NOAEL.

The Panel particularly notes the large body of evidence from RCTs which supports the UL for vitamin D of 100 μg/day. In these RCTs, doses of 100–125 μg/day were administered with or without calcium co-supplementation to various population groups for $\geq$ 12 months without evidence of persistent hypercalcaemia or hypercalciuria that could be specifically attributed to the vitamin D dose. Cases of hypercalcaemia or hypercalciuria were rare, occurred with calcium co-supplementation only, and generally resolved with supplemental calcium withdrawal. These RCTs include a wide range of the general adult population regarding age (18–90 years), sex (males and females), ethnicity (Caucasian, Asian, African American), vitamin D status (baseline serum 25(OH)D ranging from $<$ 25 to $>$ 75 nmol/L), health status (healthy free-living adults, patients recruited at the hospital, nursing home residents,

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women with osteopenia or osteoporosis), BMI (from normal weight to overweight/obese individuals) and geographical location. Furthermore, available RCTs on vitamin D supplementation at doses up to 100 μg/day for ≥ 12 months did not report an increased risk of falls or fractures, or consistent adverse effects on markers of bone health at doses up to 163 μg/day.

Based on the available evidence, chronic consumption of 100 μg/day vitamin D is not expected to pose a risk of adverse health effects. Therefore, the Panel proposes a UL of 100 μg vitamin D equivalents (VDE)/day for adults. This UL covers dietary intake of vitamin D from all sources, including fortified foods and food supplements. It applies to all forms of vitamin D authorised for addition to foods and food supplements (i.e. vitamins D_2 and D_3), and to calcidiol monohydrate. Regarding calcidiol monohydrate, the Panel notes that safety has been established up to 10 μg/day (EFSA NDA Panel, 2021a), which corresponds to 25 μg VDE/day, considering a CF for calcidiol monohydrate into vitamin D_3 of 2.5.

3.6.2.2. Pregnant and lactating women

There is no evidence that pregnancy or lactation increase the susceptibility for adverse effects of vitamin D intake. The Panel considers that the UL of 100 μg VDE/day for adults also applies to pregnant and lactating women. This UL is supported by four RCTs in pregnant or lactating women using vitamin D_3 alone or in combination with vitamin D_2 or at doses of 100 μg/day and up to 160 μg/day for 4–6 months, which did not report adverse events for either the mothers or their offspring (Hollis and Wagner, 2004; Wagner et al., 2006; Hollis et al., 2011; Enkhmaa et al., 2019). Baseline mean serum 25(OH)D concentrations widely varied across studies, from < 25 nmol/L (Enkhmaa et al., 2019) to > 75 nmol/L (Hollis and Wagner, 2004; Wagner et al., 2006).

The Panel notes that supplementation with vitamin D_3 at 100 μg/day during pregnancy and lactation in co-supplementation with calcium was associated with a higher (statistically non-significant) risk of hypercalcaemia post-partum compared to placebo in women with serum 25(OH)D concentrations < 50 nmol/L in one study (Roth et al., 2018), although it is unclear whether these cases were persistent.

3.6.2.3. Children and adolescents

Two RCTs in children aged 10–17 years have been identified in the current assessment (Maalouf et al., 2008; Rajakumar et al., 2020), one of which (Maalouf et al., 2008) was already considered in the previous risk assessment (EFSA NDA Panel, 2012b). In addition, two RCTs in children aged 9–13 years (Lewis et al., 2013) and 9–19 years (Belenchia et al., 2013) have become available. These studies show that vitamin D_3 intakes at doses of 50 μg/day and up to 100 μg/day for 6–12 months do not lead to hypercalcaemia or hypercalciuria (Lewis et al., 2013) in children and adolescents aged 10–17 years. An additional RCT reported in two publications (El-Hajj Fuleihan et al., 2006; Al-Shaar et al., 2013) conducted in children 10–17 years showed no adverse effects of vitamin D_3 at doses up to 50 μg/day for one year on markers of bone health. The Panel also considers that there is no reason to believe that adolescents in the phase of rapid bone formation and growth have a lower tolerance for vitamin D compared to adults. Thus, the Panel proposes to retain the UL for vitamin D of 100 μg VDE/day for adolescents aged 11–17 years.

For children aged 1–10 years, two RCTs have become available since the last risk assessment (EFSA NDA Panel, 2012b). One was conducted in children aged 5–15 years and provided vitamin D_2 at doses up to 178.6 μg/day for six months (Samaranayake et al., 2020) and the other was conducted in children aged 6–13 years and provided vitamin D_3 at doses up to 50 μg/day for 12 months (Asghari et al., 2021). None reported an increased risk of persistent hypercalcaemia with vitamin D supplementation. The Panel considers that there is no reason to believe that children aged 1–10 years in the phase of rapid bone formation and growth have a lower tolerance for vitamin D compared to adults and proposes to retain the UL for vitamin D of 50 μg VDE/day by considering their smaller body size.

3.7. Risk characterisation

The ULs apply to the general European population, cover dietary intake of vitamin D from all sources and apply to all forms authorised for addition to foods and food supplements (i.e. vitamins D_2 and D_3), and to calcidiol monohydrate, considering a CF for calcidiol monohydrate into vitamin D_3 of 2.5.

Harmonised data on vitamin D intake from all sources, including fortified foods and food supplements, for the European population are currently not available. Mean and high (P95) intakes of vitamin D from all sources, including fortified foods and food supplements, for all population groups were below the

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respective ULs in all the European countries which provided this information (Section 3.4.3). The Panel notes, however, that food fortification can have an important contribution to vitamin D intake, and that food fortification practices for vitamin D vary across Member States (Section 3.4.3.1).

The Panel considers that it is unlikely that the UL for vitamin D is exceeded in European populations, except for regular users of food supplements containing high doses of vitamin D.

4. Conclusions

The Panel proposes a conversion factor for calcidiol monohydrate into vitamin D_3 of 2.5 for labelling purposes.

The specific conversion factor for calcidiol monohydrate accounts for its higher efficacy in increasing serum 25(OH)D concentrations per unit dose administered as compared to cholecalciferol (vitamin D_3) for doses up to 10 μg/day.

From a scientific point of view, the Panel considers that the biological value of substances with vitamin D activity could be expressed as vitamin D equivalent (VDE), so that 1 μg VDE = 1 μg cholecalciferol (vitamin D_3) = 1 μg ergocalciferol (vitamin D_2) = 0.4 μg calcidiol monohydrate = 40 IU. This applies to calcidiol monohydrate at doses up to 10 μg/day.

The following ULs are established:

Age group	UL males and females (μg VDE/day)
0–6 months[(1)]	25
7–11 months[(1),(2)]	35
1–3 years	50
4–6 years	50
7–10 years	50
11–14 years	100
15–17 years	100
Adults (≥ 18 years)	100
Pregnant women	100
Lactating women	100

UL, Tolerable Upper Intake Level; VDE, vitamin D equivalents.
(1): Values established by the NDA Panel in 2018 (EFSA NDA Panel, 2018).
(2): Age range covers the second half of the first year of life, i.e. from the beginning of the 7th month to the 1st birthday.

5. Recommendations for research

The Panel considers that the priorities for research to inform a future revision of the UL for vitamin D are as follows:

a) To assess the relative bioavailability of equimolar doses of calcidiol monohydrate versus vitamin D_3 at doses < 20 μg VDE/day, and their effects on serum 25(OH)D concentrations, serum PTH concentrations, calcium metabolism and markers of bone health.
b) To assess the relative bioavailability of equimolar doses of vitamin D_2 versus vitamin D_3 across the whole range of intake for vitamin D and their effects on serum 25(OH)D concentrations, serum PTH concentrations, calcium metabolism and markers of bone health.
c) To elucidate the independent effect of vitamin D on long-term, persistent hypercalcaemia and hypercalciuria across the whole range of intake between the UL and the LOAEL to characterise the dose–response relationship in 'at risk' population groups, including pregnant and lactating women, ethnic groups, and the elderly.
d) To investigate the predictive value of new markers of bone quality (volumetric peripheral BMD, failure load and parameters of bone microarchitecture assessed by HR-pCT) on bone fracture risk, as well as the effect of high doses of vitamin D on these markers of bone quality in 'at risk' groups (older adults, post-menopausal women).
e) To improve knowledge about the impact of the genetic background on the biological and clinical response to vitamin D supplementation (all forms).
f) To foster ongoing efforts on the collection of accurate food composition and food consumption data on fortified foods and food supplements (i.e. vitamin D forms and concentrations/amounts).

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Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D, calcitriol
$1,24,25(OH)_3D$	1,24,25-trihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
$25(OH)D_3$	25-hydroxyvitamin D_3, calcidiol
7-DHC	7-Dehydrocholesterol
ADME	Absorption, distribution, metabolism and excretion
AI	Adequate intake
ANS Panel	Panel on Food Additives and Flavourings
AR	Average requirement
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BoE	Body of Evidence
Ca/Cr	Calcium to creatinine
CC	Case–control
CF	Conversion factor
CI	Confidence interval
CLIA	Chemiluminescence immune assay
CPBA	Competitive protein binding assay
CTx	C-telopeptide of type 1 collagen
CVD	Cardiovascular disease
D2dCA	The Vitamin D and Type 2 Diabetes Cancer Outcomes ancillary study
DBP	Vitamin D–binding protein
DRVs	Dietary reference values
DXA	Dual-energy X-ray absorptiometry
EC	European Commission
EU	European Union
EFSA	European Food Safety Authority
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FAF Panel	Panel on Food Additives and Flavourings
FCDB	EFSA Food composition database
FEEDAP Panel	Panel on Additives and Products or Substances used in Animal Feed
FGF 23	Fibroblast-growth factor 23
FIND	Finnish Vitamin D Trial
FRAX	Fracture Risk Assessment Tool
GNPD	Global New Products Database
GWAS	Genome-wide association study
HPLC	High-performance liquid chromatography
HR-pQCT	High-resolution peripheral quantitative computed tomography
HR	Hazard ratio
hs-CRP	High sensitivity C-reactive protein
IOM	Institute of Medicine
IRR	Incidence rate ratio
IU	International unit
LC–MS	Liquid chromatography–mass spectroscopy
LC–MS/MS	Liquid chromatography-tandem mass spectroscopy
LOAEL	Lowest observed adverse effect level
MS/MS	Tandem mass spectrometry
NDA Panel	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NOAEL	No observed adverse effect level
NR	Not reported
NTP	National Toxicology Program

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OHAT	Office of Health Assessment and Translation
OR	Odds ratio
PCs	Prospective cohort studies
PRI	Population reference intake
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PWV	Pulse-wave velocity
RCT	Randomised controlled trial
RoB	Risk of bias
ROM	Ratio of means
RP	Reference point
RR	Relative risk
SACN	Scientific Advisory Committee on Nutrition
SC	Scientific Committee
SCF	Scientific Committee on Food
SD	Standard deviation
sQ	Sub-question
UF	Uncertainty factor
UL	Tolerable Upper Intake Level
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
VDE	Vitamin D equivalents
VDR	Vitamin D receptor
VDSP	Vitamin D Standardisation Program
ViDA	Vitamin D and Longevity trial
VITAL	VITamin D and OmegA-3 TriaL

Glossary

Adequate intake (AI)	The value estimated when a population reference intake cannot be established because an average requirement cannot be determined. An adequate intake is the average observed daily level of intake by a population group (or groups) of apparently healthy people that is assumed to be adequate.
Adverse (health) effects	Change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity to compensate for additional stress or an increase in susceptibility to other influences (FAO/WHO, 2009; EFSA Scientific Committee, 2017).
Bioavailability	Nutrient fraction which is absorbed and becomes available to normal metabolic and physiological processes.
Biomarker of exposure	An exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism (WHO/IPCS, 1993; EFSA Scientific Committee, 2017). Urine, blood, faeces or nails are common media for the measurements of biomarkers of exposure (EFSA Scientific Committee, 2017).
Critical effect	Effect selected for the derivation of a health-based guidance value.
Dietary reference values (DRVs)	A set of nutrient reference values that includes the average requirement, the population reference intake, the adequate intake and the reference intake range for macronutrients.
Endpoint	Qualitative or quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment

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Hazard	Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population is exposed to that agent (WHO/IPCS, 2004; FAO/WHO, 2009)
Lowest-observed-adverse-effect level (LOAEL)	The lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure (FAO/WHO, 2009).
No-observed-adverse-effect level (NOAEL)	The greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure (FAO/WHO, 2009).
Tolerable Upper Intake Level (UL)	The maximum level of total chronic daily intake of a nutrient (from all sources) which is not expected to pose a risk of adverse health effects to humans.
Population reference intakes (PRI)	The level of (nutrient) intake that is enough for virtually all healthy people in a group.

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Appendix A – Flow charts for the selection of studies

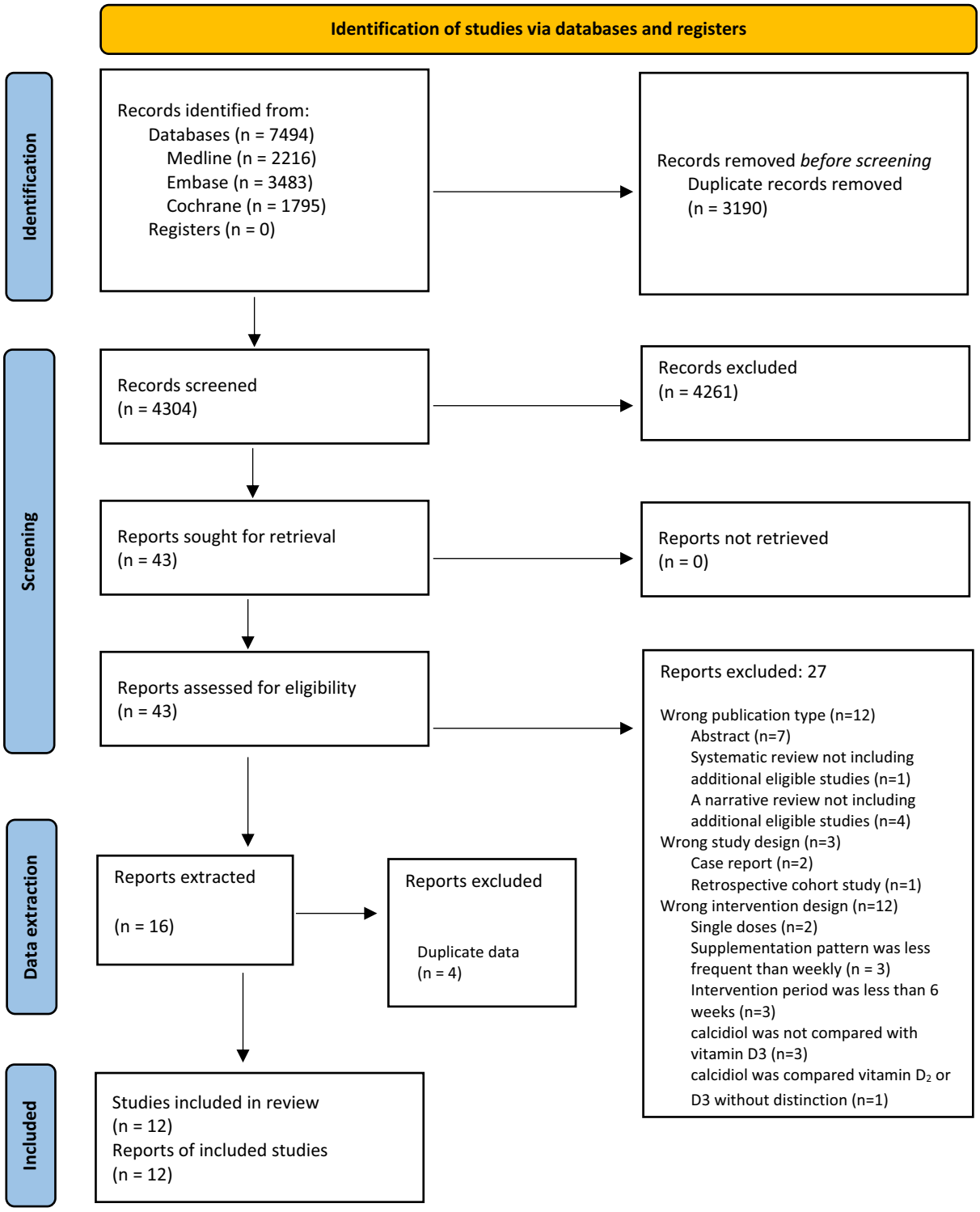

Figure A.1: Flow chart for the selection of studies on bioavailability of calcidiol and vitamin D_3

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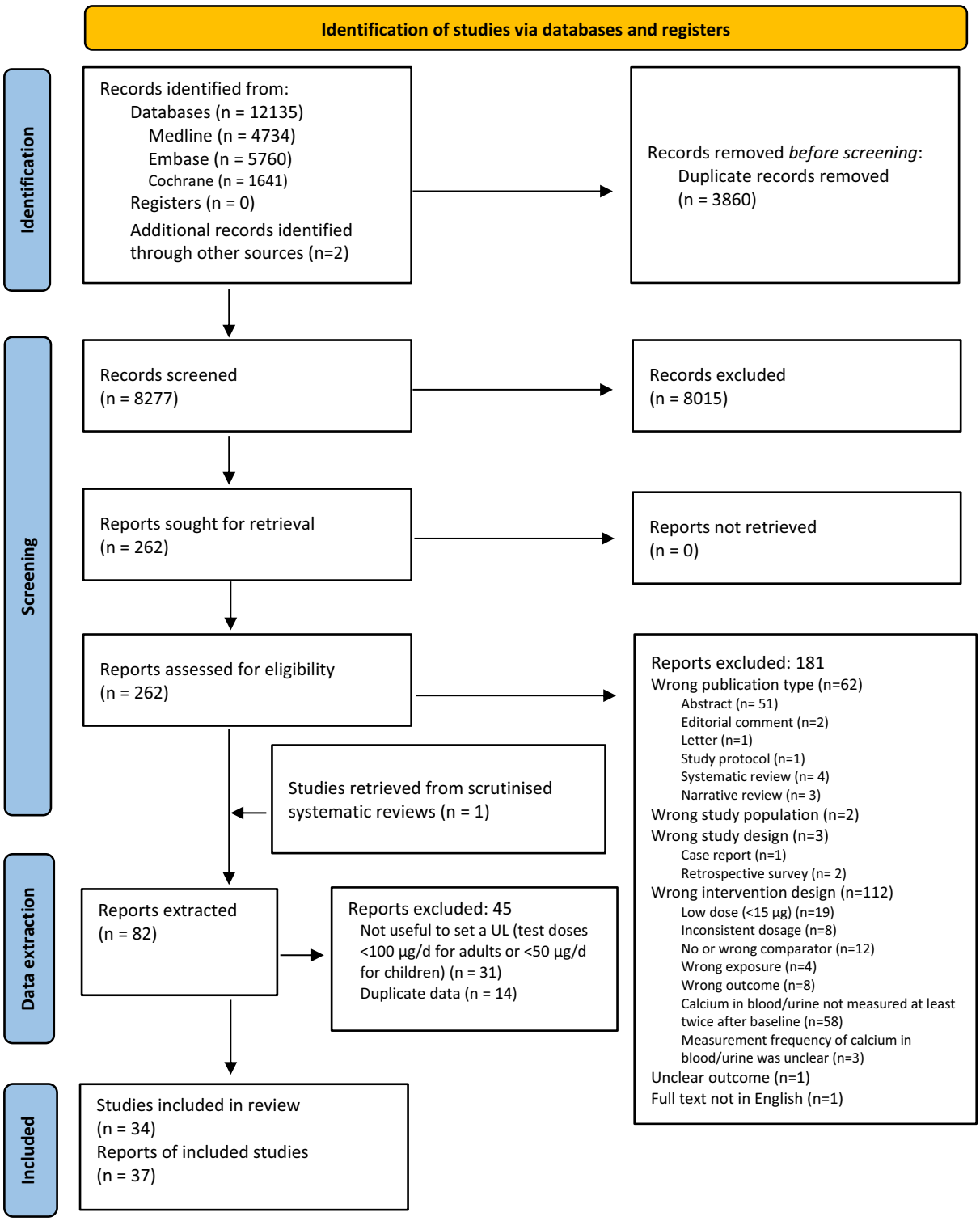

Figure A.2: Flow chart for the selection of studies on hypercalcaemia and hypercalciuria

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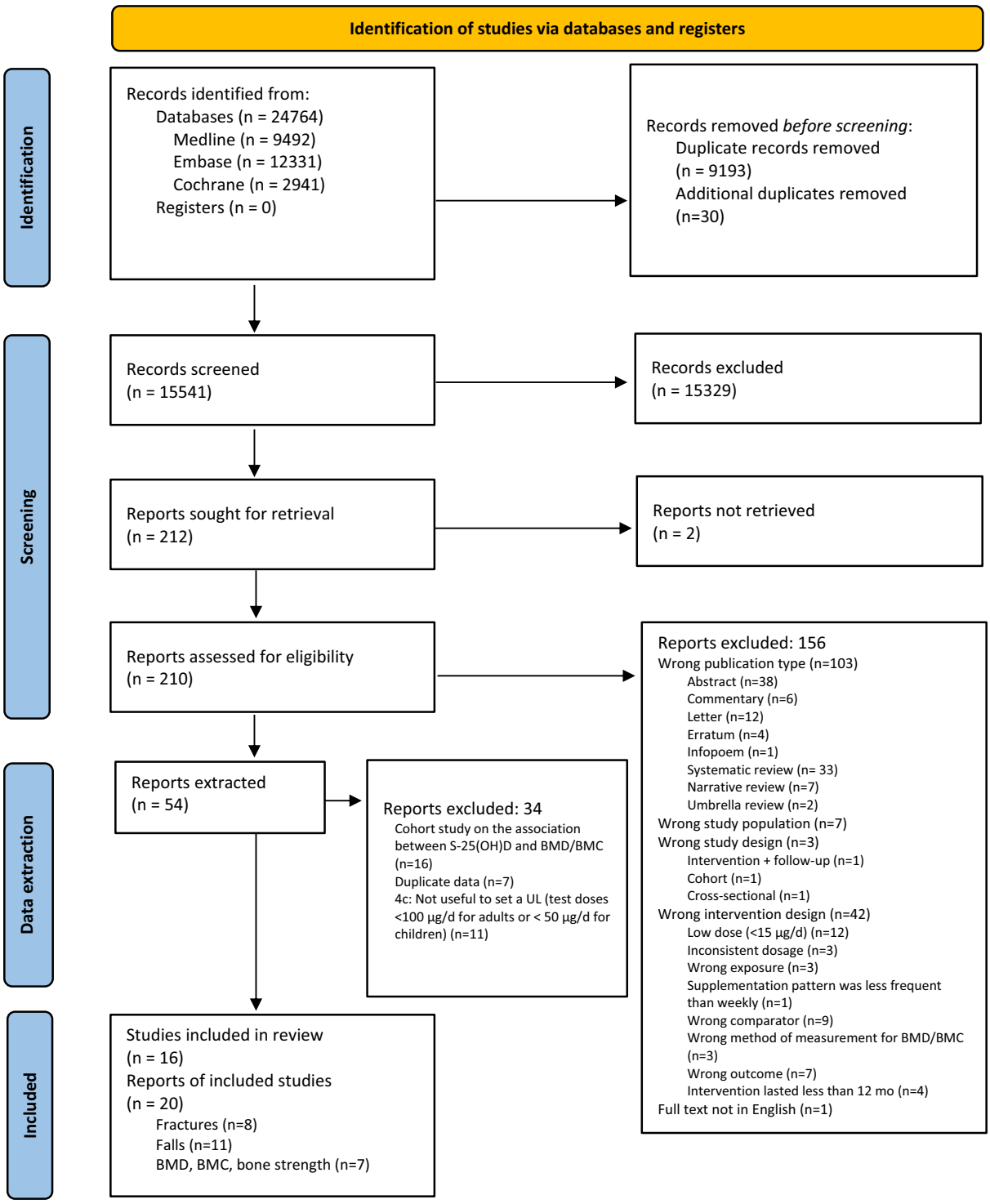

Figure A.3: Flow chart for the selection of studies on bone fractures, falls, BMD/BMC, and bone strength

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Appendix B – Outcome of the appraisal for the risk of bias of included studies

B.1. Intervention studies on bioavailability of calcidiol and vitamin D_3

References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Bischoff-Ferrari et al. 2012	+	+	+	NR	+	++	+	+	1
Cashman et al. 2012	++	++	++	++	++	++	++	++	1
Catalano et al. 2015	+	NR	NR	NR	NR	NR	++	+	3
Corrado et al. 2021	++	NR	–	NR	–	+	++	+	2
Graeff-Armas et al. 2020	++	++	+	++	++	+	++	+	1
Jakobsen et al. 2017	+	++	––	NR	+	++	++	++	2
Navarro-Valverde et al. 2016	+	NR	–	NR	––	+	++	NR	3
Okoye et al. 2022	++	––	–	–	––	––	++	+	3
Ruggiero et al. 2019	++	––	–	+	–	–	–	+	3
Shieh et al. 2017	++	++	+	NR	NR	+	++	+	1
Vaes et al. 2018a	++	++	+	++	++	++	+	+	1
Vaes et al. 2018b	++	++	+	+	++	+	+	++	1

(a): Expert judgement was translated into a rating scale for each question to be answered as follows: (++): definitely low RoB; (+): probably low RoB; (NR): not reported; (–): probably high RoB; (––): definitively high RoB.
(b): The individual rating for each question was combined by an algorithm and translated to an overall tier of reliability for each individual study (RoB tier 1: low RoB; RoB tier 2: moderate RoB; RoB tier 3: high RoB).

B.2. Intervention studies on the relationship between vitamin D intake and hypercalcaemia according to population groups

References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Children and adolescents									
Asghari et al. 2021	++	+	+	NR	––	–	–	+	2
Belenchia et al. 2013	++	+	NR	++	+	–	++	+	2
Lewis et al. 2013	++	––	+	++	+	+	++	+	2
Maalouf et al. 2008	NR	++	NR	NR	NR	+	++	+	2
Rajakumar et al. 2020	++	–	NR	++	+	–	++	+	2
Samaranayake et al. 2020	++	NR	NR	++	+	+	++	+	2
Pregnant and lactating women									
Enkhmaa et al. 2019	++	+	–	++	+	++	++	+	2
Hollis & Wagner 2004	+	+	NR	NR	NR	––	++	–	2

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References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Hollis et al. 2011	++	–	NR	+	+	–	++	+	2
Roth et al. 2018	++	–	NR	++	+	+	++	+	2
Wagner et al. 2006	++	+	NR	++	++	– –	++	+	2
General adult population									
Aloia et al. 2013	++	–	+	++	++	–	++	++	2
Aloia et al. 2018	++	+	+	NR	NR	–	++	+	2
Billington et al. 2020	++	–	+	++	+	+	++	++	2
Brohult & Jonson, 1973	+	NR	NR	NR	–	–	++	–	3
Burnett-Bowie et al. 2012	++	+	NR	NR	NR	++	++	+	2
Diamond et al. 2013	++	NR	NR	NR	– –	NR	++	+	3
Drincic et al. 2013	++	++	–	NR	– –	+	++	+	2
Gallagher et al. 2012[c]	++	++	+	++	+	+	++	++	1
Gallagher et al. 2013[c]	++	+	+	+	+	+	++	+	1
Grimnes et al. 2012	++	++	+	++	+	++	++	++	1
Heaney et al. 2003	+	NR	–	NR	NR	NR	++	+	3
Hin et al. 2016	++	–	+	++	+	++	++	+	2
Johnson et al. 2022	++	+	–	+	++	++	+	+	2
Sneve et al. 2008	++	+	NR	NR	+	–	++	+	2
Mastaglia et al. 2006	+	+	NR	–	–	+	++	+	2
Ponda et al. 2012	++	NR	NR	++	+	NR	+	+	2
Rafii et al. 2019	NR	+	–	– –	– –	–	+	–	3
Rorie et al. 2014	++	+	NR	++	+	+	++	+	2
Schwartz et al. 2016	++	++	NR	NR	+	+	–	+	2
Shirvani et al. 2020	++	NR	NR	NR	+	NR	++	+	2
Vieth et al. 2001	+	+	+	++	+	–	+	+	1
Wagner et al. 2016	++	NR	+	++	++	++	++	+	2
Wamberg et al. 2013	++	+	+	++	+	+	+	+	1

(a): Expert judgement was translated into a rating scale for each question to be answered as follows: (++): definitely low RoB; (+): probably low RoB; (NR): not reported; (−): probably high RoB; (− −): definitively high RoB.
(b): The individual rating for each question was combined by an algorithm and translated to an overall tier of reliability for each individual study (RoB tier 1: low RoB; RoB tier 2: moderate RoB; RoB tier 3: high RoB).
(c): The publications were based on the same intervention study but represent different study populations.

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B.3. Intervention studies on relationship between vitamin D intake and hypercalciuria according to population groups

References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Children and adolescents									
Lewis et al. 2013	++	−−	++	++	+	+	++	+	2
Lactating women									
Hollis & Wagner 2004	+	+	NR	NR	NR	−−	++	−	2
Hollis et al. 2011	++	−	−	+	+	−	++	+	2
Wagner et al. 2006	++	+	NR	++	++	−−	++	+	2
General adult population									
Aloia et al. 2013	++	−	++	++	++	−	++	++	2
Aloia et al. 2018	++	+	++	NR	NR	−	++	+	2
Billington et al. 2020	++	−	+	++	+	+	++	++	2
Ceglia et al. 2013	NR	NR	++	+	+	+	++	+	2
Gallagher et al. 2012[c]	++	++	++	++	+	+	++	++	1
Gallagher et al. 2013[c]	++	+	+	+	+	+	++	+	1
Johnson et al. 2022	++	+	−	+	++	++	+	+	2
Mastaglia et al. 2006	+	+	−	−	−	+	++	+	2
Rafii et al. 2019	NR	+	−	−−	−−	−	+	−	3
Rorie et al. 2014	++	+	NR	++	+	+	++	+	2
Vieth et al. 2001	+	+	+	++	+	−	+	+	1

(a): Expert judgement was translated into a rating scale for each question to be answered as follows: (++): definitely low RoB; (+): probably low RoB; (NR): not reported; (−): probably high RoB; (−−): definitively high RoB.
(b): The individual rating for each question was combined by an algorithm and translated to an overall tier of reliability for each individual study (RoB tier 1: low RoB; RoB tier 2: moderate RoB; RoB tier 3: high RoB).
(c): The publications were based on the same intervention study but represent different study populations.

B.4. Intervention studies on relationship between vitamin D intake and fractures

References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Bischoff-Ferrari et al. 2020	++	+	++	++	++	+	++	+	1
Burt et al. 2019	++	+	++	++	+	++	++	+	1
Flicker et al. 2005	++	−−	+	++	+	−−	++	+	2
Grant et al. 2005	++	−	+	++	+	−−	++	+	2
Hin et al. 2016	++	+	−−	++	−	++	++	+	2

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References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Macdonald et al. 2013	++	++	+	++	+	–	++	++	1
Peacock et al. 2020	++	+	+	NR	+	–	++	+	1
Prince et al. 2008	++	+	NR	++	+	+	++	++	2

(a): Expert judgement was translated into a rating scale for each question to be answered as follows: (++): definitely low RoB; (+): probably low RoB; (NR): not reported; (–): probably high RoB; (––): definitively high RoB.
(b): The individual rating for each question was combined by an algorithm and translated to an overall tier of reliability for each individual study (RoB tier 1: low RoB; RoB tier 2: moderate RoB; RoB tier 3: high RoB).

B.5. Intervention studies on relationship between vitamin D intake and falls

References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criterion					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Appel et al. 2021	––	–	+	++	+	+	++	+	2
Bischoff-Ferrari et al. 2022	++	–	++	++	++	+	++	+	2
Burt et al. 2019	++	+	++	++	+	++	++	+	1
Flicker et al. 2005	++	––	+	++	+	––	++	+	2
Grant et al. 2005	++	–	+	++	+	––	++	+	2
Hin et al. 2016	++	+	––	++	–	++	++	+	2
LeBoff et al. 2020a	++	–	–	NR	+	NR	++	+	2
Prince et al. 2008	++	+	NR	++	+	+	++	++	2
Smith et al. 2017	++	++	––	++	+	NR	++	–	2
Uusi-Rasi et al. 2015	++	+	++	++	++	+	++	++	1
Wood et al. 2014	++	++	––	++	+	+	++	++	2

(a): Expert judgement was translated into a rating scale for each question to be answered as follows: (++): definitely low RoB; (+): probably low RoB; (NR): not reported; (–): probably high RoB; (––): definitively high RoB.
(b): The individual rating for each question was combined by an algorithm and translated to an overall tier of reliability for each individual study (RoB tier 1: low RoB; RoB tier 2: moderate RoB; RoB tier 3: high RoB).

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B.6. Intervention studies on relationship between vitamin D intake and BMD, BMC, and Bone Strength

References	Risk of bias domains[a]								Tier[b]
	Key Criteria			Other Criteria					
	Randomization	Exposure characterization	Outcome assessment	Allocation concealment	Blinding	Attrition	Selective reporting	Other threats to internal validity	
Children and adolescents[c]									
Al-Shaar et al. 2013 - Females	++	++	+	++	+	++	+	+	1
Al-Shaar et al. 2013 - Males	++	NR	+	++	+	++	–	–	2
El-Hajj Fuleihan et al. 2006 - Females	++	++	+	++	+	++	+	+	1
El-Hajj Fuleihan et al. 2006 – Males	++	NR	+	++	+	++	– –	– –	2
General adult population									
Burt et al. 2019	++	+	++	++	+	++	++	+	1
Grimnes et al. 2012	++	+	++	++	+	++	++	+	1
Jorde et al. 2010	++	+	+	NR	+	–	++	+	1
Rahme et al. 2017	++	++	+	++	++	+	++	++	1
Smith et al. 2018	++	++	++	++	+	+	+	+	1

(a): Expert judgement was translated into a rating scale for each question to be answered as follows: (++): definitely low RoB; (+): probably low RoB; (NR): not reported; (–): probably high RoB; (––): definitively high RoB.
(b): The individual rating for each question was combined by an algorithm and translated to an overall tier of reliability for each individual study (RoB tier 1: low RoB; RoB tier 2: moderate RoB; RoB tier 3: high RoB).
(c): All study groups were based on the same intervention study.

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Appendix C – Evidence tables for the studies included

C.1. Intervention studies on the relative bioavailability of calcidiol [25(OH)D_3] compared to native vitamin D_3

Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
Bischoff-Ferrari et al. (2012) Switzerland Latitude [assumed] 47.4° N 4 mo Private funding	RCT (parallel) Inclusion criteria: White postmenopausal women aged 50–70, in general good health, with serum 25(OH)D level between 8–24 ng/mL [20–60 nmol/L], BMI 18–29 kg/m^2, and ability of giving informed consent. Exclusion criteria: Medical contraindications to vitamin D supplements, medical conditions, or medication use that would alter pharmacokinetics of study products or otherwise interfere with the study; specific exclusion criteria are listed in Appendix 1 that included e.g. current smokers; use of dietary supplements while on study, except multivitamins, must stop before entering the study and refrain from using until the end of the	Sex: Females Age (y) G1–G2: 63.45 ± 7.78 G3–G4: 59.48 ± 6.27 Serum 25(OH)D (ng/mL) G1–G2: 14.18 ± 3.61 ng/mL [35.45 ± 9.03 nmol/L] G3–G4: 12.28 ± 4.08 ng/mL [30.70 ± 10.2 nmol/L] iPTH (pg/mL) G1–G2: 54.87 ± 10.71 G3–G4: 63.22 ± 16.37 BMI (kg/m^2) G1–G2: 25.49 ± 3.38 G3–G4: 23.24 ± 3.22 Ethnicity: White Smoking status: Smokers were excluded Alcohol use: NR Health status: Healthy population. Season: NR	Doses G1: vitamin D_3 20 μg/d G2: vitamin D_3 140 μg/wk [=20 μg/d] G3: calcidiol 20 μg/d G4: calcidiol 140 μg/wk [=20 μg/d] The daily and weekly groups for each vitamin D form were combined for the analysis as the results between daily and weekly supplementations did not differ significantly. Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method: Complete adherence stated.	Serum 25(OH)D: measured by HPLC–MS/MS • time of blood collectionafter last dosage: NR iPTH: measured by ECLIA	Data extracted: unclear Serum 25(OH)D at 4 mo (ng/mL), mean ± SE G1–G2: 30.99 ± 1.59 ng/mL [77.48 ± 3.98 nmol/L] G3–G4: 69.47 ± 1.58 ng/mL [173.68 ± 3.95 nmol/L] iPTH at 4 mo (pg/mL), mean ± SE G1–G2: 51.68 ± 3.43 G3–G4: 43.00 ± 3.43	**1**

Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
	study; use of high-dose vitamin D supplements >400 IU [>10 μg]; use of high-dose calcium supplements >600 mg. N participants randomised/completed/analysed G1–G2: 10 October 2010 G3–G4: 10 October 2010					
Cashman et al. (2012) Republic of Ireland Latitude 51° N 10 wk Mixed funding	RCT (parallel) Inclusion criteria: White men and women aged 50 years or more. Exclusion criteria: If unwilling to discontinue the consumption of vitamin D–containing supplements 8 wk before the initiation of the study and throughout the study; if planning to take a winter vacation (during the course of the 10-wk intervention) to a location at which either the altitude or the latitude was predicted to result in significant cutaneous vitamin D synthesis from solar radiation (eg, a winter sun coastal resort or a mountain ski resort); if using tanning facilities of	Sex (% females) G1: 63 G2: 62 G3: 50 G4: 42 Age (y) All: 57.2 ± 6.3 NR across groups. Serum 25(OH)D (nmol/L) G1: 42.7 ± 12.6 G2: 49.7 ± 16.2 G3: 42.5 ± 8.9 G4: 38.2 ± 9.9 Serum iPTH (ng/mL) G1: 65.6 (47.4–70.2) G2: 47.3 (41.5–57.5) G3: 58.6 (52.8–69.9) G4: 57.9 (42.5–73.5) BMI (kg/m^2) All: 28.3 ± 4.8 NR across groups. Ethnicity: White	Doses G1: placebo G2: vitamin D_3 20 μg/d G3: calcidiol 7 μg/d G4: calcidiol 20 μg/d Background vitamin D intake (μg/d) G1: 6.5 (2.9–7.9) G2: 7.6 (2.9–5.4) G3: 5.1 (2.8–6.6) G4: 4.4 (3.7–6.1) Background calcium intake (mg/d) G1: 970 ± 503 G2: 1114 ± 494 G3: 1008 ± 415 G4: 794 ± 309 Compliance Pill count method (%) All: 97.0 (92.0–100). Stated to be similar across groups (P = 0.9).	Serum 25(OH)D: measured by ELISA (OCTEIA) Serum iPTH: measured by ELISA	Data extracted: PP Serum 25(OH)D at 5 wk/during intervention (nmol/L) G1: 39.7 ± 11.1 G2: 64.1 ± 9.5 G3: 60.8 ± 8.1 G4: 98.1 ± 20.5 Serum 25(OH)D at 10 wk (nmol/L) G1: 41.2 ± 11.1 G2: 69.0 ± 8.7 G3: 70.7 ± 9.9 G4: 134.6 ± 26.0 Serum iPTH at 10 wk (ng/mL) G1: 65.8 (54.5–87.8) G2: 44.2 (40.1–52.7) G3: 52.7 (41.1–62.7) G4: 40.5 (34.6–61.6)	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
	any type; a severe medical illness, hypercalcaemia, known intestinal malabsorption syndrome, excessive alcohol use, use of medications known to interfere with vitamin D metabolism. N participants randomised/completed/analysed G1: 16/16/16 G2: 14/13/13 G3: 14/14/14 G4: 14/13/12	Smoking status: NR Alcohol use: Excessive alcohol users were excluded. Health status: Apparently healthy population. Season: winter; 17–28th Jan to 21st Mar–8th Apr				
Catalano et al. (2015) Italy Latitude [assumed] 38.2° N 24 wk Unclear funding	RCT (parallel) Inclusion criteria: Postmenopausal women; osteopenic in accordance with WHO criteria; without prevalent vertebral or nonvertebral fractures, and with a low fracture risk according to a validated fracture risk assessment tool; assuming a stabilized (at least 6 mo) HMG-CoA reductase therapy with atorvastatin prescribed as appropriate at a dosage of 20 or 40 mg/day and if they showed serum 25 (OH)D levels <30 ng/mL [< 75 nmol/L].	Sex: Females Age (y) G1: 59.46 ± 6.12 G2: 58.62 ± 7.35 Serum 25(OH)D (ng/mL) G1: 20.35 ± 5.46 ng/mL [50.88 ± 13.65 nmol/L] G2: 22.30 ± 6.58 ng/mL [55.75 ± 16.45 nmol/L] Serum PTH: NR BMI (kg/m^2) G1: 26.79 ± 2.45 G2: 27.48 ± 2.81 Ethnicity [assumed]: Majority Caucasian Smoking status (%) Current smoking:	Doses G1: vitamin D_3 140 μg/wk [=20 μg/d] G2: calcidiol 140 μg/wk [=20 μg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance NR	Serum 25(OH)D: method of measurement NR • time of blood collectionafter last dosage: NR Serum PTH: not measured	Data extracted: unclear Serum 25(OH)D at 24 wk (ng/mL) G1: 24.30 ± 7.82 ng/mL [60.75 ± 19.55 nmol/L] G2: 50.33 ± 15.45 ng/mL [125.83 ± 38.63 nmol/L] Serum PTH at 10 wk: NR	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
	Exclusion criteria: Cancer, liver or renal failure, hypo- or hypercalcaemia, and previous treatment with intravenous N-BPs or previous (within 6 months) hormone replacement therapy or assumption of selective oestrogen receptor modulators. N participants randomised/completed/analysed G1: 28/NR/28 G2: 29/NR/29	G1: 28 G2: 31 Alcohol use: NR Health status (%) Hypertension: G1: 28 G2: 34 Diabetes: G1: 36 G2: 44 Season: Enrolment period Sept to Dec 2012.				
Corrado et al. (2021) Italy Latitude [assumed] 40.6° N 6 mo Unclear funding [stated no external funding received]	RCT (parallel) Inclusion criteria: Postmenopausal women with serum 25(OH)D level between 8 ng/mL to 24 ng/mL [20 to 60 nmol/L], BMI between 18 and 29 kg/m^2 and good health. Exclusion criteria: Taking drugs which could interfere with calcium and phosphate homeostasis; taking antihypertensive drugs, bisphosphonates, anticonvulsant, anticoagulant,	Sex: Females Age (y) G1: 63.4 ± 5.5 G2: 60.9 ± 8.1 Serum 25(OH)D (ng/mL) G1: 12.5 ± 2.46 [31.25 ± 6.15 nmol/L] G2: 13.3 ± 2.9 [33.25 ± 7.25 nmol/L] Serum PTH (units unclear) G1: 35.5 (4.1) G2: 28.3 (8.08) BMI (kg/m^2) G1: 23.8 ± 1.5 G2: 23.3 ± 1.2 Ethnicity: White	Doses G1: vitamin D_3 7000 IU/wk [175 μg/wk = 25 μg/d] G2: calcidiol 7000 IU/wk [175 μg/wk = 25 μg/d] The design also included two other groups treated with single dose of 300 000 IU [=7500 μg] of vitamin D_3 and monthly dose of 100 000 IU [=2500 μg] of vitamin D_3. The groups were ignored here as supplementation pattern	Serum 25(OH)D: measured by CLIA (Diasorin LIASON kit [accredited VDSP]) • time of blood collectionafter last dosage: NR Serum PTH: measured by EIA	Data extracted: unclear Serum 25(OH)D_3 at 6 mo (nmol/L) G1: 50.9 ± 4.52 [127.25 ± 11.3 nmol/L] G2: 66.8 ± 3.98 [167.25 ± 9.95 nmol/L] Serum PTH at 6 mo (units unclear) G1: 34.2 (7.7) G2: 31.2 (6.7)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
	corticosteroid, thiazide diuretics, hormone therapy, estrogen receptor modulators for 6 months prior to recruitment; diseases that can alter the vitamin D intestinal absorption; diseases with increased risk of hypercalcaemia (sarcoidosis, lymphoma, primary hyperparathyroidism), kidney stones, intestinal malabsorption (celiac disease, lactose intolerance, gastric resection), severe renal impairment, psychiatric disorder, motor disability; potentially unable to properly take the vitamin D supplementation and unable to perform muscular exercises; the occurrence of fractures in the last year or fall in the 3 mo before; high intensity exercise, abuse of alcohol, cigarettes, intensive sun exposure in the 3 mo before the study.	Smoking status: Cigarettes as an exclusion criterion. Alcohol use: Abusers of alcohol were excluded. Health status: Good health. Season: Oct to Apr.	was less frequent than weekly. Background vitamin D intake: NR Background calcium intake: NR Compliance NR			

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
	N participants randomised/completed/analysed G1: 31/27/27 G2: 30/26/26					
Graeff-Armas et al. (2020) United Kingdom Latitude 51° N 6 mo Mixed funding	RCT (parallel) Inclusion criteria: Healthy Caucasian men and postmenopausal women aged >50 y, with a BMI of 20–32 kg/m^2 and willing to avoid direct sun exposure and restrict travel to sunny climates during the study period. Exclusion criteria: Taking vitamin D supplements (≤2 mo since last vitamin D supplement use), taking >500 mg/d of calcium from supplements; a history of hypercalcaemia, malabsorption, kidney or liver disease, acute or severe illness; planning a beach holiday, using a tanning bed, or heavy consumption of alcohol; being on steroids, anticonvulsants, antipsychotics, antibiotics, estrogen replacement, bisphosphonate, or	Sex (% females) G1: 59 G2: 64 G3: 61 G4: 50 Age (y) G1: 65.1 ± 9.3 G2: 62.8 ± 8.4 G3: 62.5 ± 6.8 G4: 63.0 ± 7.3 Serum 25(OH)D_3 (nmol/L) G1: 47.1 ± 15.0 (n = 21) G2: 48.0 ± 17.3 G3: 49.5 ± 17.2 G4: 48.5 ± 17.2 Serum iPTH (pmol/L) G1: 3.91 ± 1.28 G2: 4.12 ± 1.34 G3: 3.81 ± 1.11 G4: 3.99 ± 1.11 BMI (kg/m^2) G1: 26.4 ± 3.1 G2: 26.1 ± 2.3 G3: 27.1 ± 3.1 G4: 25.4 ± 2.5 Ethnicity: Caucasian Smoking status: NR	Doses [labeled] G1: vitamin D_3 20 µg/d G2: calcidiol 10 µg/d G3: calcidiol 15 µg/d G4: calcidiol 20 µg/d Analysed doses G1: vitamin D_3 20.8 µg/d G2: calcidiol 10.8 µg/d G3: calcidiol 15.6 µg/d G4: calcidiol 21.8 µg/d Background vitamin D intake (µg/d): NR Background calcium intake (mg/d): NR Compliance Pill count method (%) > 97 on average across groups and all visits.	Serum 25(OH)D_3: measured by LC–MS/MS Serum iPTH: measured by CLIA (Roche Cobas; Roche)	Data extracted: PP Serum 25(OH)D_3 at 6 mo (nmol/L) G1: 82.8 ± 18.1 (n = 21) G2: 103 ± 22.9 (n = 19) G3: 130 ± 20.5 G4: 153 ± 30.6 Serum iPTH at 6 mo (pmol/L) G1: 4.77 ± 1.86 G2: 4.31 ± 1.55 G3: 4.00 ± 0.98 G4: 4.00 ± 0.84 (n = 21)	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
	recombinant PTH treatment or any drug that would alter gastrointestinal fat absorption. N participants randomised/completed/analysed G1: 22[1)]/22/22 G2: 22[1)]/20/21 G3: 23[1)]/23/23 G4: 24[1)]/23/24 [1)] received intervention; two of the randomised participants did not receive treatment due to being unable to cannulate.	Alcohol use: Heavy alcohol users were excluded. Health status: Healthy Season: 12th to 20th Nov at baseline.				
Jakobsen et al. (2017) Denmark Latitude 55° N 3 x 6 wk Unclear funding	RCT (crossover) Inclusion criteria: Healthy, free-living male adults aged 20 to 30 y. Exclusion criteria: BMI > 27 kg/m^2, had donated blood within the last 3 mo, any chronic diseases, use of medication regularly except for the occasional use of painkillers; were hypercalcemic, excessive consumption of alcohol, or known malabsorption syndromes.	Sex: Males Age (y): 23 ± 3 Serum 25(OH)D (nmol/L): 56.1 ± 8.5 Serum iPTH (pmol/L): 3.2 ± 1.3 BMI (kg/m^2): 23.2 ± 2 Ethnicity: Caucasian Smoking status: Non-smokers	Doses G1: vitamin D_2 10 µg/d G2: vitamin D_3 10 µg/d G3: calcidiol 10 µg/d Analysed doses G1: vitamin D_2 10.2 µg G2: vitamin D_3 9.9 µg G3: calcidiol 9.8 µg Run-in: vitamin D_3 10 µg/d for 4 wk. Wash-out: No Background vitamin D intake (µg/d): 1.1 ± 0.4 Measured 3 times during the intervention.	Serum 25(OH)D: measured by LC–MS/MS Serum iPTH: measured by CLIA	Data extracted: unclear Observed levels Serum 25(OH)D after 6 wk (nmol/L) G1: 44.2 ± 8.0 G2: 55.1 ± 8.9 G3: 64.7 ± 11.2 Serum iPTH after 6 wk (pmol/L) G1: 2.8 ± 1.0 G2: 2.1 ± 0.7 G3: 2.4 ± 0.9 Estimated levels, mean (95% CI) Serum 25(OH)D after 6 wk (nmol/L)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
	N participants randomised/completed/analysed G1: 12 December 2012 G2: 12 December 2012 G3: 12 December 2012	Alcohol use: Excessive alcohol users were excluded. Health status: Healthy Season: Mid-Oct 2006 to Mar 2007.	Background calcium intake (mg/d): 806 ± 361 Measured 3 times during the intervention. Compliance Pill count method (%): 97		G1: 43.5 (40.9, 46.4) G2: 54.4 (51.1, 58.0) G3: 63.8 (59.9, 67.9) Serum iPTH after 6 wk (pmol/L) G1: 2.6 (2.2, 3.0) G2: 2.0 (1.7, 2.4) G3: 2.2 (1.9, 2.6) Models included the factors treatment and period (P = 0.3–0.8), the covariate the baseline value, and a random effect of person.	
Navarro-Valverde et al. (2016) Spain Latitude 36.7° N 12 mo Unclear funding	RCT (parallel) Inclusion criteria: NR; was reported that post-menopausal osteopenic women (diagnostic criteria NR) with good health, average age 67 years, and deficient in vitamin D (serum 25(OH)D 37.5 ± 5 nmol/L) were studied. Exclusion criteria: NR N participants randomised/completed/analysed G1: 10/NR/NR G2: 10/NR/NR G3: 10/NR/NR	Sex: Females Age (y) All: 67 ± 6 NR across groups. Serum 25(OH)D (nmol/L) G1: 40.5 ± 4.7 G2: 37.2 ± 4.2 G3: 38.0 ± 3.7 Serum PTH (ng/L) All: 57.2 ± 11.0 NR across groups. BMI (kg/m^2) All: 26.4 ± 4 NR across groups. Ethnicity [assumed]: Majority Caucasian Smoking status: NR Alcohol use: NR Health status:	Doses G1: vitamin D_3 20 μg/d G2: calcidiol 20 μg/d G3: calcidiol 266 μg/wk [=38 μg/d] The design also included a group treated with fortnightly dose of 266 μg [=19 μg/d] of calcidiol. The group was ignored as supplementation pattern was less frequent than weekly. Background vitamin D intake: NR Background calcium intake: NR Compliance NR	Serum 25(OH)D: measured by HPLC and ultraviolet detection method • time of blood collectionafter last dosage: NR Serum PTH: measured by ECLIA (Roche Diagnostic)	Data extracted: unclear Serum 25(OH)D at 6mo (nmol/L) G1: 80.0 ± 2.0 G2: 161 ± 21.7 G3: 213.5 ± 80.0 Serum 25(OH)D at 12 mo (nmol/L) G1: 86.2 ± 23.7 G2: 188.0 ± 24.0 G3: 233.0 ± 81.2 Serum 25(OH)D, change over 12 mo (nmol/L) G1: 45.7 ± 19.5 G2: 150.8 ± 22.3 G3: 195 ± 79.3 Serum PTH at 6 mo (ng/L) G1: 46.5 ± 11.2 G2: 38.7 ± 10.7 G3: 35.4 ± 8.4 Serum iPTH at 12 mo (ng/L) G1: 41.6 ± 10.5 G2: 32.5 ± 8.8 G3: 29.0 ± 5.9	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
		Osteoporotic or osteopenic women? Former stated in abstract, latter stated in the methods; method of diagnosis NR Season: NR				
Okoye et al. (2022) Italy Latitude [assumed] 43.7° N From hospital admission to 3 mo after discharge. Unclear funding [stated no external funding received]	RCT (parallel) Inclusion criteria: Geriatric patients consecutively hospitalized in the Geriatric Unit of the University Hospital of Pisa for acute illness, with 25(OH)D_3 levels <30 ng/mL [<75 nmol/L]. Exclusion criteria: Having received vitamin D supplementation during the past 6 mo; stage V renal insufficiency; liver failure (defined as a Child–Pugh classification of a B or C); hyperparathyroidism; malabsorption syndromes or the long-term prescription of drugs reducing vitamin D absorption (i.e., antiepileptic drugs, long-term corticosteroids, or bisphosphonates); neoplastic disease under	Sex (% females) G1: 56.5 G2: 59.1 Age (y): G1: 84.9 ± 6.4 G2: 82.7 ± 6.7 Plasma 25(OH)D_3 (ng/mL) G1: 16.8 ± 9.9 ng/mL [42.0 ± 24.75 nmol/L] G2: 18.8 ± 13.3 ng/mL [47.0 ± 33.25 nmol/L] PTH (ng/dL) G1: 48.1 ± 39.6 G2: 60.7 ± 36.9 BMI (kg/m^2) G1: 23.7 (7.2) G2: 25.0 (5.6) Ethnicity [assumed]: Majority Caucasian Smoking status: NR Alcohol use: NR Health status (%) Arterial hypertension: G1: 69.6	Doses G1: vitamin D_3 437.5 μg/wk [=62.5 μg/d] G2: calcidiol 140 μg/wk [=20 μg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method: Complete adherence stated.	Plasma 25(OH)D_3: measured by HPLC–MS/MS • time of blood collectionafter last dosage: NR PTH: method of measurement NR	Data extracted: unclear Plasma 25(OH)D_3 at 3 mo (ng/mL) G1: 30.7 ± 8.4 ng/mL [76.75 ± 21.0 nmol/L] G2: 45.4 ± 9.8 ng/mL [113.5 ± 24.5 nmol/L] PTH at 3 mo (ng/dL): NR	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
	treatment; patients being unable to give informed consent. N participants randomised/completed/ analysed G1: 89/69/69 G2: 89/70/71	G2: 70.4 Coronary heart disease: G1 11.6 G2: 9.9 Atrial fibrillation: G1: 17.4 G2: 19.7 Heart failure: G1: 42.0 G2: 39.4 Diabetes: G1: 20.3 G2: 21.1 Chronic kidney disease: G1: 23.2 G2: 22.5 Chronic obstructive pulmonary disease: G1: 8.6 G2: 9.8 Season: May to Sept 2020				
Ruggiero et al. (2019) Italy Latitude [assumed] 43.1° N From hospital admission to 7 mo after discharge. Unclear funding [stated no external funding received]	RCT, pragmatic (parallel) Inclusion criteria: Community-dwelling women and men, oldest-old patients, aged >75 years, consecutively admitted to geriatric acute care ward. Exclusion criteria: On treatment with vitamin D, multivitamins, calcium supplements,	Sex (% females) G1: 61 G2: 65 Age (y) G1: 82.0 (77.0–86.0) G2: 83.5 (79.8–86.5) Serum 25(OH)D (ng/mL) G1: 10 (7–14) ng/mL [25 (17.5–35) nmol/L] G2: 10 (4–16) ng/mL [25 (10–40) nmol/L] Serum iPTH (pg/mL)	Doses G1: vitamin D_3 150 μg/wk [=21.43 μg/d] G2: calcidiol 150 μg/wk [=21.43 μg/d] During hospital stay participants received 1000 mg/d calcium supplements; at discharge they received recommendations to assure adequate calcium intake from diet	Serum 25(OH)D: measured by RIA (Diasorin) • time of blood collectionafter last dosage: NR Serum iPTH: measured by EIA	Data extracted: unclear Serum 25(OH)D at 7 mo (ng/mL) G1: NR; increased by 16 ng/mL [40 nmol/L] on average G2: NR; increased by 19 ng/mL [47.5 nmol/L] on average. Serum iPTH (pg/mL) at 7 mo G1: 53.7 ± 13.6 G2: 58.9 ± 13.9	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
	anti-fracture drugs or steroids; suffered from fragility fractures within 6 mo, cancer within 5 y, hyperparathyroidism, hypercalcaemia, hypercalciuria, hypophosphatemia, Paget's disease, chronic renal failure, nephrolithiasis, bowel inflammatory diseases, bowel resection, malabsorption syndrome, including celiac and Crohn's diseases, liver disease, excessive alcohol use, tuberculosis or sarcoidosis; major surgical within 6 mo, unable to walk outdoors before admission, were bedridden, nursing home residents at the time of admission or become eligible for nursing home placement during the course of the hospital stay, or if participating in other clinical studies. N participants randomised/completed/analysed G1: 33/NR/NR G2: 34/NR/NR	G1: 50 (38–85) or 64.1 ± 38.2 G2: 104 (47–145) or 99.6 ± 51.2 BMI (kg/m^2) G1: 27.5 (25.9–29.4) G2: 26.8 (24.4–28.6) Ethnicity[assumed]: Majority Caucasian Smoking status: NR Alcohol use: Excessive alcohol users were excluded. Health status: Number of comorbidities G1: 7 (5–10) G2: 7 (5–9) Season: NR	and, eventually, from supplements. Background vitamin D intake: NR Background calcium intake: NR <u>Compliance</u> Strategies to prove compliance were not implemented.			

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
Shieh et al. (2017) USA Latitude [assumed] 34.05° N 16 wk Mixed funding	RCT (parallel) Inclusion criteria: Age ≥ 18 years and a baseline serum 25(OH)D level < 20 ng/mL [<50 nmol/L] Participants agreed to refrain from changing their dietary calcium intake and from taking self-prescribed calcium or vitamin D supplements for the study duration. Exclusion criteria: History of hypercalcaemia, hypercalciuria, nephrolithiasis, intestinal malabsorption, or dysregulated vitamin D metabolism (from underlying comorbidity or medication). N participants randomised/completed/analysed G1: 16/NR/NR G2: 19/NR/NR	Sex: NR Age (y) G1: 36.9 ± 12.7 G2: 34.8 ± 8.6 Serum 25(OH)D (ng/mL) G1: 16.2 ± 3.7 [40.5 ± 9.25 nmol/L] G2: 17.0 ± 2.5 [42.5 ± 6.25 nmol/L] Serum iPTH (pg/mL) G1: 40.1 ± 18.6 G2: 34.6 ± 13.9 BMI (kg/m^2) G1: 25.7 ± 6.1 G2: 27.4 ± 7.4 Ethnicity (%) White: G1: 12.5 G2: 15.8 African American: G1: 37.5 G2: 26.3 Asian American: G1: 37.5 G2: 31.6 Hispanic/Latino: G1: 12.5 G2: 26.3 Smoking status: NR Alcohol use: NR Health status: Healthy Season: NR	Doses G1: vitamin D_3 60 µg/d G2: calcidiol 20 µg/d Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method (%) G1: 90.1 on average G2: 91.9 on average	Serum 25(OH)D: measured by CLIA (Diasorin LIAISON) Serum iPTH: measured by CLIA (Cobas; Roche)	Data extracted: unclear Serum 25(OH)D at 16 wk (ng/mL) G1: 29.6 ± 4.1 [74.0 ± 10.25 nmol/L] G2: 42.4 ± 15.9 [106.0 ± 39.75 nmol/L] Serum iPTH (pg/mL) at 16 wk: NR	1

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
Vaes et al. (2018a) the Netherlands Latitude 51° N 6 mo Mixed funding	RCT (parallel) Inclusion criteria: 65 years or older, with a serum 25(OH)D level between 20 and 50 nmol/L and a BMI between 18.5 and 35 kg/m^2; prefrail or frail based on the frailty criteria of Fried et al (2001). Exclusion criteria: A serum calcium level >2.6 nmol/L or uncontrolled hypocalcemia, diagnosed malabsorption disorders, sarcoidosis, lymphoma, primary hyperparathyroidism, kidney stones (in the past 10 y), renal insufficiency, cancer, the use of medication that may influence vitamin D metabolism [e.g., bisphosphonates, parathyroid hormone (PTH) treatment, anti-tuberculosis medications, anti-epilepticmedications, bile acid sequestrate, or lipase inhibitors]; consumption of >21	Sex (% females) G1: 46 G2: 42 G3: 46 Age (y) G1: 73.7 ± 6.2 G2: 74.8 ± 6.7 G3: 73.1 ± 6.0 Serum 25(OH)D (nmol/L), mean (95% CI) 2) G1: 38.1 (32.5, 43.8) G2: 36.3 (30.6, 42.0) G3: 38.1 (32.5, 43.8) Plasma iPTH (pmol/L), mean (95% CI)2) G1: 6.5 (5.6, 7.4) G2: 7.6 (6.7, 8.5) G3: 7.5 (6.6, 8.4) BMI (kg/m^2) G1: 27.8 ± 3.7 G2: 27.4 ± 3.6 G3: 27.6 ± 3.5 Ethnicity[assumed]: Majority Caucasian Smoking status: NR Alcohol use: Those consuming >21 servings alcoholic beverages/wk were excluded. Health status (%)	Doses [labeled] G1: placebo G2: vitamin D_3 20 µg/d G3: calcidiol 10 µg/d Analysed doses G1: placebo G2: vitamin D_3 22.9 µg/d G3: calcidiol 9.9 µg/d Background vitamin D intake (µg/d) G1: 3.6 ± 1.5 G2: 3.6 ± 1.1 G3: 3.5 ± 1.6 Background calcium intake (mg/d) G1: 1010 ± 555 G2: 985 ± 304 G3: 1110 ± 481 Compliance Pill count method (%) All: 98 on average.	Serum 25(OH)D: measured by LC–MS/MS (The Vitamin D external quality certified laboratory) Plasma iPTH: measured by sandwich CLIA	Data extracted: ITT? Stated that analyses were performed on the basis of the intention-to-treat principle. Serum 25(OH)D at 6 mo (nmol/L), mean (95% CI) G1: 47.5 (41.8, 53.3) G2: 72.0 (66.1, 77.8) G3: 98.7 (93.1, 104.4) Serum 25(OH)D, change over 3 mo (nmol/L), mean (95% CI) 2) G1: 9.6 (2.6, 16.5) G2: 31.8 (24.8, 38.9) G3: 54.0 (47.1, 60.8) Serum 25(OH)D, change over 6 mo (nmol/L), mean (95% CI) 2) G1: 8.9 (2.0, 15.9) G2: 35.7 (28.6, 42.7) G3: 60.6 (53.7, 67.5) Plasma iPTH, change over 3 mo (pmol/L), mean (95% CI) 2) G1: 0.1 (−0.7, 0.9) G2: −1.0 (−1.8, −0.2) G3: −2.0 (−2.8, −1.2) Plasma iPTH, change 6 mo (pmol/L), mean (95% CI) 2) G1: 0.3 (−0.5, 1.1) G2: −1.4 (−2.2, −0.6) G3: −1.7 (−2.5, −0.9) 2) model-adjusted means; ”Covariates (age, sex, and BMI) were included on the basis of model fit”	1

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results[a]	RoB Tier
	servings of alcoholic beverages/wk; not willing or able to stop the use of vitamin D–containing supplements during the study; expected to increase their sun exposure (e.g., planned holiday), or had a surgery planned. N participants randomised/completed/analysed G1: 26/25/25 G2: 26/24/24 G3: 26/26/26	Prefrail: G1: 96 G2: 96 G3: 81 Frail: G1: 4 G2: 4 G3: 19 Season: Dec 2014 to Dec 2015. 2) model-adjustedmeans				
Vaes et al. (2018b) the Netherlands Latitude 51° N 24 wk Private funding	RCT (parallel) Inclusion criteria: 65 years or older; a serum 25(OH)D_3 concentration between 25 and 50 nmol/L and a BMI between 20 and 35 kg/m^2. Exclusion criteria: A serum calcium level >2.6 mmol/L, diagnosis with kidney stones in the past 10 years, renal insufficiency, liver failure, malabsorption syndromes, sarcoidosis and primary hyperparathyroidism; use of medication that might	Sex (% females) G1: 64 G2: 43 G3: 40 G4: 44 Age (y) G1: 78 ± 7.7 G2: 80 ± 7.3 G3: 79 ± 7.0 G4: 80 ± 7.0 Serum 25(OH)D_3 (nmol/L) G1: 37.7 ± 7.0 G2: 43.4 ± 15.8 G3: 38.3 ± 10.5 G4: 38.6 ± 12.9 iPTH (pmol/L) G1: 5.2 ± 1.9 G2: 5.7 ± 1.7	Doses [labeled] G1: vitamin D_3 20 µg/d G2: calcidiol 5 µg/d G3: calcidiol 10 µg/d G4: calcidiol 15 µg/d Analysed doses G1: vitamin D_3 22.3 µg/d G2: calcidiol 5.1 µg/d G3: calcidiol 10.3 µg/d G4: calcidiol 15.3 µg/d Background vitamin D intake (µg/d) G1: 3.7 ± 1.2 G2: 4.2 ± 1.6 G3: 3.3 ± 1.3 G4: 3.5 ± 1.5 Background calcium intake (mg/d)	Serum 25(OH)D_3: all samples were measured at the end of the study; measured by LC–MS/MS iPTH: measured by sandwich CLIA	Data extracted: PP Serum 25(OH)D_3 at 24 wk (nmol/L), mean (95% CI) 4) G1: 71.6 (63.2, 80.0) G2: 52.2 (44.4, 60.2) G3: 88.7 (81.4, 96.1) G4: 109.9 (102.5, 117.2) iPTH at 24 wk (pmol/L), mean (95% CI) 4) G1: 4.7 (4.1, 5.2) G2: 5.1 (4.6, 5.6) G3: 4.8 (4.3, 5.3) G4: 3.9 (3.4, 4.4) 4) model-predicted means; " The baseline level of the response variable and BMI were included as covariates in all models."	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
	interfere with vitamin D metabolism led to exclusion (e.g. thiazides, parathyroid hormone, bisphosphonates); consumed >3 alcoholic beverages per day, used vitamin D supplements in the 3 months prior to the screening visit, not willing to stop the use of multivitamins during the study, expected to increase sun exposure (e.g. planned holiday to a sunny resort), being blood donor or had a surgery planned. N participants randomised/completed/ analysed G1: 14[3)]/12/11 G2: 14[3)]/13/12 G3: 15[3)]/15/14 G4: 16[3)]/14/14 [3)] received intervention; one of the randomised participants did not receive treatment due to violation of eligibility criteria.	G3: 4.9 ± 1.3 G4: 4.9 ± 1.8 BMI (kg/m^2) G1: 27.6 ± 3.5 G2: 26.0 ± 4.4 G3: 26.6 ± 3.7 G4: 26.8 ± 3.9 Ethnicity [assumed]: Majority Caucasian Smoking status (%) Non-smokers: G1: 43 G2: 36 G3: 40 G4: 31 Ex-smokers: G1: 50 G2: 64 G3: 60 G4: 69 Current smokers: G1: 7 G2: 0 G3: 0 G4: 0 Alcohol use (%) Light: G1: 71 G2: 79 G3: 93 G4: 75 Moderate: G1: 29 G2: 21	G1: 985 ± 438 G2: 1204 ± 487 G3: 1041 ± 293 G4: 1111 ± 386 Compliance Pill count method (%) All: 97 on average.			

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results[(a)]	RoB Tier
		G3: 7 G4: 25 Excessive: G1: 0 G2: 0 G3: 0 G4: 0 Health status: NR Season: 26th Aug 2013 to 30th Apr 2014.				

Abbreviations: CI: confidence interval; CLIA: chemiluminescence immune assay; CT: controlled trial; ECLIA: electrochemiluminescence immune assay; EIA: enzyme immunoassay; ELISA: enzyme-linked immunosorbent assay; G: group; HPLC–MS/MS: high-performance liquid chromatography tandem mass spectrometry; iPTH: intact parathyroid hormone; ITT: intention-to-treat; LC–MS/MS: liquid chromatography tandem mass spectrometry; NR: not reported; PP: per-protocol; PTH: parathyroid hormone; RCT: randomised controlled trial; RIA: radioimmunoassay; SE: standard error; 25(OH)D: 25-hydroxyvitamin D.

(a): the values have been reported as mean ± standard deviation or median (inter quartile range) unless otherwise indicated; interquartile range = 25^{th}–75^{th} percentiles or the length between the percentiles.

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C.2. Intervention studies on the persistent hypercalcaemia and/or hypercalciuria

Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Children and adolescents						
Asghari et al. (2021) Iran Latitude 35.7° N 1 yr Unclear [No external funding]	RCT (parallel) Inclusion criteria: Age 6–13 y; age- and sex-specific BMI Z-score ≥1. Exclusion criteria: Serious illness, vitamin D supplement use. N participants, randomised/completed/ analysed: G1: 120/100 at 6mo, 92 at 12mo/120 G2: 127/106 at 6mo, 101 at 12mo/127 G3: 131/112 at 6mo, 108 at 12mo/131	Sex (% girls) G1: 50 G2: 48 G3: 45 Age (y) G1: 9.2 ± 1.8 G2: 9.4 ± 1.6 G3: 9.4 ± 1.8 Serum 25(OH)D (ng/mL): G1: 11.5 (8.9) ng/mL [28.8 (22.3) nmol/L] G2: 11.7 (10.5) ng/mL [29.3 (26.3) nmol/L] G3: 12.3 (10.2) ng/mL [30.8 (25.3)] Assay: ECLIA (electrochemiluminescence immunoassay (Roche Diagnostics) BMI (kg/m^2) G1: 23.2 ± 3.0 G2: 23.5 ± 3.5 G3: 23.3 ± 3.5 Ethnicity [assumed]: Persian Smoking status: NR Alcohol use: NR	Vitamin D_3 Doses (labeled) G1: vitamin D_3 4 × 1000 IU/wk => 600 IU/d / 15 μg/d G2: vitamin D_3 1000 IU/d / 25 μg/d G3: vitamin D_3 2000 IU/d / 50 μg/d Analysed vitamin D_3 doses G2: 25.1 μg/d G3: 51.1 μg/d Background vitamin D intake (IU/d): G1: 30.8 (71.6) IU/d [0.8 (1.8) μg/d] G2: 43.2 (94.4) [1.1 (2.4) μg/d] G3: 32.8 (86.0) [0.8 (2.2) μg/d] Background calcium intake (mg/d): G1: 574 (353) G2: 605 (432) G3: 610 (454) Compliance	Serum calcium: - secondary outcome - measured at baseline and at 6 and 12 mo - no cut-off for elevated levels provided.	Stated that no side effect was reported in any intervention groups, no increase in concentration of calcium was observed and intervention did not have any effect on serum calcium. Serum calcium at baseline (mg/dl), median (IQR) G1: 9.9 (9.5–10.4) G2: 10.1 (9.7–10.6) G3: 9.9 (9.5–10.4) Serum calcium at 6 mo (mg/dl), median (IQR) G1: 10.4 (10.1–10.7) G2: 10.3 (10–10.6) G3: 10.5 (10–10.7) Serum calcium at 12 mo (mg/dl), median (IQR) G1: 10.4 (10.1–10.7) G2: 10.3 (10.1–10.7) G3: 10.3 (10.1–10.7)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
		Health status: Overweight and obese Season at recruitment (%) Spring and summer: G1: 50.8 G2: 50.4 G3: 49.6 Autumn and winter: G1: 49.2 G2: 49.6 G3: 50.4	Pill count method – ≥80% of pills consumed (%): G1: 84.3 (76.9–91.8) G2: 87.5 (78.5–96.0) G3: 84.0 (76.3–92.6) Serum 25(OH)D (ng/mL) at 12 mo: G1: 23.11 (18.55–26.52) ng/mL [57.78 (46.38–66.3) nmol/L] G2: 25.56 (21.27–29.58) ng/mL [63.9 (53.18–73.95) nmol/L] G3: 28.63 (24.55–34.97) ng/mL [71.58 (61.38–87.43) nmol/L]			
Belenchia et al. (2013) USA Latitude [assumed] 38.9° N 6 mo Private funding	RCT (parallel) Inclusion criteria: Aged 9 to 19 y; being at least at the 85th percentile for BMI. Exclusion criteria: 1) use of vitamin D supplements other than a general multivitamin; 2) the use of medication affecting vitamin D metabolism; 3) the use of a tanning bed or undergoing UV light therapy; 4) use of oral hypoglycemic agents; 5) previously diagnosed	Sex (% Females) G1: 52 G2: 48 Age (y) G1: 13.9 ± 2.4 G2: 14.6 ± 2.3 Serum 25(OH)D (ng/mL) G1: 19.6 ± 7.9 [49 ± 19.75 nmol/L] G2: 19.2 ± 6.3 [48 ± 15.75 nmol/L] Assay: ELISA (Immunodiagnostik AG) BMI (kg/m^2) G1: 38.9 ± 6.7 G2: 39.5 ± 5.1	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 4000 IU/d [100 μg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method (%) G1: 82 G2: 81 Serum 25(OH)D at 6 mo	Serum calcium: - secondary outcome - measured at baseline and at 3 and 6 mo - no cut-off for elevated levels provided.	Serum calcium concentrations remained unchanged in both groups throughout the study and were well within the normal range at each time point.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	hepatic or renal disorders; 6) pregnancy; 7) or the use of tobacco or alcohol. N participants, randomised/completed/ analysed: G1: 23/15/17 G2: 21/14/18	Ethnicity (%) African American: G1: 26 G2: 33 Other ethnicities: NR Smoking status: NR Alcohol use: NR Health status: Obese Season: Recruitment occurred from Nov 2009 until Jan 2011.	G1: no significant change from baseline G2: significant increase from baseline. Changes independent of season.			
Lewis et al. (2013) USA Latitude 34° N and 40° N 12 wk Private funding	(RCT parallel) Inclusion criteria: Children at sexual maturity stages 2 and 3. Both parents and grandparents were the same race as the child and considered themselves non-Hispanic. Children taking nutritional supplements were enrolled after a 4-week washout. Children agreed to not alter dietary or physical activity pat Exclusion criteria: Included menarche, growth disorders, diseases (eg, cerebral palsy), and medications (eg, corticosteroids) known to influence bone metabolism.	Sex (% Females) All: 49.8 Age (y) G1: 11.5 ± 1.2 G2: 11.3 ± 1.2 G3: 11.1 ± 1.1 G4: 11.4 ± 1.4 G5: 11.5 ± 1.2 Serum 25(OH)D (nmol/L) G1: 71.5 ± 18.6 G2: 71.4 ± 19.5 G3: 71.1 ± 19.7 G4: 65.8 ± 7.3 G5: 70.0 ± 17.5 Assay: 2-step RIA (Diasorin) and further monitored through DEQAS BMI-for-age percentiles G1: 63.3 (29.5) G2: 67.6 (27.8) G3: 70.4 (28.4)	Vitamin D_3 Doses (labeled) G1: placebo G2: vitamin D_3 400 IU/d [10 μg/d] G3: vitamin D_3 1000 IU/d [25 μg/d] G4: vitamin D_3 2000 IU/d [50 μg/d] G5: vitamin D_3 4000 IU/d [100 μg/d] Analysed doses G1: 0.184 IU [0.0046 μg] G2: 486 IU [12.15 μg] G3: 1140 IU [28.5 μg] G4: 1880 IU [47 μg] G5: 4710 IU [117.75 μg]	Hypercalcaemia: - safety measure - blood samples were collected at baseline and at 3, 6, 9, and 12 wk, and calcium was apparently analysed - defined serum calcium >10.6 mg/dL[2.625 mmol/L]. Hypercalciuria: - safety measure - second-void urine samples were collected at each visit - defined as urine calcium corrected for creatinine >0.22 mg [0.055 mmol].	**Hypercalcaemia** Over 12 wk, 3 children met the criteria defining hypercalcaemia, and none of these children were assigned to 100 μg/d. **Hypercalciuria** Over 12 wk, 3 children met the criteria defining hypercalciuria, and none of these children were assigned to 100 μg/d.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	N participants, randomised/completed/ analysed: G1: 66/63/63 G2: 64/59/59 G3: 65/63/63 G4: 64/61/61 G5: 64/58/58	G4: 71.5 (30.4) G5: 67.4 (30.1) Ethnicity (%) White: 48.9 Black: 51.1 Smoking status: NR Alcohol use: NR Health status: Healthy children Season: Enrolment in 2009 to 2010 and 2010 to 2011 during the winter (Oct through Dec) when serum 25(OH)D is at its nadir.	Background vitamin D intake (IU/d) G1: 151 ± 96 IU/d [3.8 ± 2.4 µg/d] G2: 198 ± 140 IU/d [5.0 ± 3.5 µg/d] G3: 143 ± 111 IU/d [3.6 ± 2.8 µg/d] G4: 184 ± 160 IU/d [4.6 ± 4.0 µg/d] G5: 175 ± 101 IU/d [4.4 ± 2.5 µg/d] Background calcium intake (mg/d) G1: 837 ± 321 G2: 1000 ± 467 G3: 822 ± 375 G4: 914 ± 411 G5: 945 ± 378 Compliance Pill count method – vitamin D_3/placebo (%) All: 52.3 Serum 25(OH)D, change over 12 wk (nmol/L), mean ± SE G1: −10.12 ± 2.86 G2: 5.54 ± 2.59 G3: 20.29 ± 2.61 G4: 37.57 ± 2.66 G5: 76.07 ± 2.95			
Maalouf et al. (2008)	RCT (parallel) Inclusion criteria:	Sex (%) Females: 49.4	Vitamin D_3 Doses (labeled)	Hypercalcaemia: - safety measure	Elevated serum calcium, *n* of participants	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
Lebanon Latitude [assumed] 33.9° N 1 year Private funding	Age 10–17 y. Exclusion criteria: Disorders or medications known to affect bone metabolism N participants, randomised/completed/analysed Females: G1: NR/NR/55 G2: NR/NR/58 G3: NR/NR/55 Males: G1: NR/NR/56 G2: NR/NR/56 G3: NR/NR/60	Boys: 50.6 Age (y) All: 13.1 ± 2 Serum 25(OH)D (ng/mL): Females: G1: 14 ± 7 [35 ± 17.5 nmol/L] G2: 14 ± 9 [35 ± 22.5 nmol/L] G3: 13 ± 8 [32.5 ± 20 nmol/L] Males: G1: 16 ± 6 [40 ± 15 nmol/L] G2: 16 ± 7 [40 ± 17.5 nmol/L] G3: 16 ± 7 [40 ± 17.5 nmol/L] Assay: RIA (Diasorin) BMI: NR Ethnicity [assumed]: Arab Smoking status: NR Alcohol use: NR Health status: Apparently healthy Season: Summer to early fall	G1: placebo G2: vitamin D_3 200 IU/d [5 μg/d] G3: vitamin D_3 2000 IU/d [50 μg/d] Analysed doses: Stated that the vitamin D concentration in the three solutions was within 10% of that anticipated based on the label on the bottles and the dilution protocol. Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – intake of the total dose of vitamin D (%) G1: 98 ± 3 G2: 98 ± 3 G1: 97 ± 3 Serum 25(OH)D at 1 year (ng/mL) Females: G1: 16 ± 8 [40 ± 20 nmol/L]	- serum calcium measured at baseline and at 6 and 12 mo - defined as >10.7 mg/dl [2.675 mmol/L].	G1: 2 girls (10.8 and 11.1 mg/dl [2.7 and 2.78 mmol/L]) and 3 boys (10.8 mg/dl [2.7 mmol/L]) G2: 1 boy (10.9 mg/dl [2.72 mmol/L]) G3: 1 boy (11.0 mg/dl [2.75 mmol/L]) Stated that the 7 children had serum calcium levels above the upper limit of normal at 1 yr.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
			G2: 17 ± 6 [42.5 ± 15 nmol/L] G3: 38 ± 31 [95 ± 77.5 nmol/L] Males: G1: 17 ± 6 [42.5 ± 15 nmol/L] G2: 20 ± 7 [50 ± 17.5 nmol/L] G2: 35 ± 9 [87.5 ± 22.5 nmol/L]			
Rajakumar et al. (2020) USA Latitude [assumed] 40.4° N 6 mo Mixed funding	RCT (parallel) Inclusion/exclusion criteria: Overweight or obese children (BMI ≥85th percentile) who were free of conditions or treatments that could affect glucose homeostasis, BP, cholesterol concentrations, or vitamin D and calcium metabolism. Eligible participants were vitamin D–deficient (serum 25-hydroxyvitamin D [25(OH)D] <20 ng/mL) and had normal serum calcium (10–14 y: 8.8–10.8 mg/d [2.2–2.7 mmol/L]; ≥15 y, 8.4–10.2 [2.1–2.55 mmol/L] mg/dL) during a screening assessment and	Sex (% Females) G1: 71.1 G2: 60.8 G3: 64.0 Age (y) G1: 13.5 ± 2.3 G2: 13.5 ± 2.2 G3: 13.9 ± 2.4 Serum 25(OH)D (ng/mL) G1: 14.3 ± 4.3 [35.8 ± 10.8 nmol/L] G2: 14.4 ± 3.4 [36.0 ± 8.5 nmol/L] G3: 14.2 ± 3.5 [35.5 ± 8.8 nmol/L] Assay: LC–MS/MS BMI (kg/m^2) G1: 30.7 ± 6.9 G2: 30.0 ± 6.1 G3: 30.3 ± 5.8 Ethnicity (%) Black:	Vitamin D_3 Doses (labeled) G1: vitamin D_3 600 IU/d [15 μg/d] G2: vitamin D_3 1000 IU/d [25 μg/d] G3: vitamin D_3 2000 IU/d [50 μg/d] Analysed doses G1: 754 IU [18.85 μg] G2: 1086 IU [27.15 μg] G3: 2142 IU [53.55 μg] Background vitamin D intake (IU/d) G1: 192 (124–288) [4.8 (3.1–7.2) μg/d] G2: 197 (121–285) [4.9 (3.0–7.1) μg/d] G3: 189 (122–331) [4.7 (3.1–8.3) μg/d]	Hypercalcaemia: - safety measure - laboratory data were obtained at enrolment and at 3 and 6 mo, and calcium was apparently analysed (see table 1) - normal serum calcium defined as 8.8–10.8 mg/dL [2.2–2.7 mmol/L] (10–14 y) and 8.4–10.2 mg/dL [2.1–2.55 mmol/L] (15 y)	The vitamin D doses were tolerated without development of hypercalcaemia.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	who had fasting glucose concentrations of <125 mg/dL and, in the case of postmenarchial girls, had a negative urine pregnancy test at the time of randomization. N participants, randomised/completed/ analysed: G1: 76/50/50 G2: 74/48/47 G3: 75/58/58	G1: 93.4 G2: 94.6 G3: 93.3 Hispanic: G1: 2.6 G2: 1.4 G3: 9.3 Smoking status: NR Alcohol use: NR Health status: Overweight or obese vitamin D–deficient children Season: NR	Background calcium intake (mg/d) G1: 905 (701–1287) G2: 987 (647–1374) G3: 913 (599–1391) Compliance Pill count method and validated by an electronic medication event monitoring system – vitamin D_3 (%) All: 73 (at 3 and 6 mo) At 3 mo G1: 73 G2: 68 G3: 77 At 6 mo G1: 73 G2: 73 G3: 73			
Samaranayake et al. (2020) Sri Lanka Latitude [assumed] 6.9° N 24 wk Public funding	(RCT parallel) Inclusion criteria: Obese children, who are identified to be having vitamin D deficiency (defined as 25(OH) D < 20 ng/ml or < 50 nmol/L) at a previously conducted cross-sectional study Exclusion criteria: Children who were suffering from any other	Sex (% Females) G1: 19.4 G2: 33.3 G3: 32.3 Age (y) G1: 10.61 ± 1.83 G2: 9.75 ± 2.26 G3: 9.95 ± 2.02 Serum 25(OH)D (ng/mL): G1: 15.47 ± 2.78 ng/mL [38.68 ± 6.95 nmol/L] G2: 14.92 ± 3.92 ng/mL [37.30 ± 9.80 nmol/L]	Vitamin D_2 Doses G1: placebo G2: vitamin D_2 2500 IU/ wk [62.5 μg/ wk = 8.9 μg/d] G3: vitamin D_2 50 000 IU/wk [1250 μg/ wk = 178.6 μg/d] Background vitamin D intake: NR Background calcium intake: NR	Hypercalcaemia - safety measure - serum calcium measured at baseline, at 12 wk, and 6 mo - no cut-off provided	No adverse events were reported in the study population. Mean serum calcium decreased significantly in each group.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	chronic condition or were on long-term medication were excluded. N participants, randomised/completed/ analysed: G1: 31/27/31 G2: 33/25/33 G3: 32/27/32	G3: 14.92 ± 3.04 ng/mL [37.30 ± 7.60 nmol/L] Assay: NR BMI Z-score G1: 2.66 ± 0.55 G2: 2.83 ± 0.86 G3: 2.72 ± 0.65 Ethnicity (%) Sinhala: G1: 74.2 G2: 69.7 G3: 64.5 Tamil: G1: 10.5 G2: 9.1 G3: 12.9 Muslim G1: 16.1 G2: 21.2 G3: 19.4 Smoking status: NR Alcohol use: NR Health status: Obese children Season: NR	Compliance Participants kept a written record of their compliance with the treatment. During the follow up visits the subjects were assessed for compliance to treatment Stated: Compliance was found to be satisfactory in all the subjects who completed the trial. Serum 25(OH)D at 24 wk (ng/mL): G1: 15.77 ± 3.43 ng/mL [39.43 ± 8.58 nmol/L] G2: 15.26 ± 3.675 ng/mL [38.15 ± 9.19 nmol/L] G3: 18.24 ± 5.77 ng/mL [45.60 ± 14.43 nmol/L]			
Pregnant and lactating women						
Enkhmaa et al. (2019) Mongolia	RCT (parallel) Inclusion criteria: Pregnant women; ≥18 y;	Sex: Females Age (y) G1: 28.3 ± 5.6	Vitamin D_3 Doses	Hypercalcaemia: - safety measure	There were no instances of hypercalcaemia in any study arm at any timepoint.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Latitude 50° N From 12–16 weeks' gestation to 36–40 weeks' gestation Mixed funding	12–16 weeks pregnant; planning to deliver at Mandal Soum Hospital; willing to forego vitamin D supplements other than the study capsules. Exclusion criteria: Current known seizure disorder, renal failure, parathyroid disease, thyroid disease, sarcoidosis, cancer, or tuberculosis; history of kidney stones; known sensitivity to multivitamin preparations; already taking vitamin D supplements containing >600 IU/day [15 μg/d]. N participants, randomised/completed/analysed: G1: 119/109/119 G2: 121/111/121 G3: 120/113/120	G2: 28.5 ± 5.7 G3: 28.5 ± 5.4 Serum 25(OH)D (nmol/L) G1: 18 ± 21 G2: 20 ± 24 G3: 20 ± 22 Assay: VIDAS® enzyme linked fluorescent assay (ELFA, Biomérieux) Weight status (%) Underweight (<18.5 kg/m^2): G1: 0 G2: 1 G3: 0 Normal weight (18.5–24.9 kg/m^2) G1: 40 G2: 42 G3: 54 Overweight (25–29.9 kg/m^2): G1: 40 G2: 42 G3: 29 Obese (≥30 kg/m^2): G1: 20 G2: 15 G3: 17 Ethnicity [assumed]: Mongol Smoking status (%) Never: G1: 94 G2: 93	G1: vitamin D_3 600 IU/d [15 μg/d] + calcium 300 mg/d G2: vitamin D_3 2000 IU/d [50 μg/d] + calcium 300 mg/d G3: vitamin D_3 4000 IU/d [100 μg/d] + calcium 300 mg/d Vitamin D_3 content was validated by Covance Laboratories (Madison, WI) using LC–MS to ensure the capsules met label claims at the outset of the study. Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – taking at least 80% of their assigned vitamin D supplements (%) G1: 88 G2: 89 G3: 87 Serum 25(OH)D at 36–40 weeks' gestation (nmol/L) G1: 46 ± 21	- serum calcium measured at 2 mo and at 36–40 weeks of gestation - defined as >2.6 mmol/L / 10.4 mg/dl In any instances of hypercalcaemia, the Data Safety and Monitoring Board was to be immediately informed and the participant was to discontinue the supplement until the calcium concentrations could be retested. Any woman with confirmed hypercalcaemia was to be withdrawn from the study.		

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
		G3: 97 Past: G1: 6 G2: 5 G3: 3 Current: G1: 0 G2: 2 G3: 0 Alcohol use: NR Health status: NR Season at baseline (%) Winter: G1: 24 G2: 22 G3: 23 Spring: G1: 35 G2: 36 G3: 37 Summer: G1: 19 G2: 22 G3: 21 Fall: G1: 21 G2: 21 G3: 20	G2: 70 ± 23 G3: 81 ± 29			
Hollis and Wagner (2004) USA Latitude [assumed] 32° N	(RCT parallel) Inclusion criteria: Fully lactating mothers within 1 month after birth were eligible for inclusion	Sex: Females Age (y) G1: 29.0 ± 6.0 G2: 30.8 ± 5.2	Vitamin D_2 + D_3 Doses G1: vitamin D_2 1600 IU/d [40 µg/d] + vitamin	Serum calcium and urinary Ca/Cr ratio: - measured at months 1, 2, 3, and 4 of lactation - no cut-off for elevated levels provided	Serum calcium concentrations all remained in the normal range.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
3 mo Unclear funding	in the study if they planned to continue full breastfeeding for the next 3 mo. Exclusion criteria: Preexisting type 1 or type 2 diabetes mellitus, hypertension, parathyroid disease, and uncontrolled thyroid disease. N participants, randomised/completed/ analysed: All: 64 (enrolled)/18/18 G1: NR/9/9 G2: NR/9/9	Serum 25(OH)D (ng/mL), mean ± SEM G1: 27.6 ± 3.3 ng/mL [69.0 ± 8.3 nmol/L] G2: 32.9 ± 2.4 ng/mL [82.3 ± 6.0 nmol/L] Assay: HPLC and RIA techniques BMI (kg/m^2): NR Ethnicity (%) African American: G1: 33.3 G2: 22.2 White G1: 66.6 G2: 77.8 Smoking status: NR Alcohol use: NR Health status: Healthy adults Season: NR	D_3 400 IU/d [10 μg/d] G2: vitamin D_2 3600 IU/d [90 μg/d] + vitamin D_3 400 IU/d [10 μg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – vitamin D3 (%) Month 1 G1: 89.7 G2: 91.6 Month 2 G1: 87.9 G2: 89.6 Month 3 G1: 89.8 G2: 92.4 Serum 25(OH)D at 3 mo (ng/mL) G1: 36.1 ± 2.3 ng/mL [90.3 ± 5.8 nmol/L] G2: 44.5 ± 3.9 ng/mL [111.3 ± 9.8 nmol/L]		No observation of hypercalciuria was noted.	
Hollis et al. (2011) USA	(RCT parallel) Inclusion criteria: (1) maternal age of 16 years or greater at the	Sex: Females Age (y) G1: 27.0 ± 5.6 G2: 27.4 ± 5.7	Vitamin D_3 Doses G1: placebo + vitamin D_3 400 IU/d [10 μg/d]	Serum calcium - collected at each visit (monthly study visits, which continued until delivery)	It was stated that throughout the study, there were no statistically significant differences between groups on any	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Latitude [assumed] 32° N 12–16 weeks of gestation until delivery Public funding	time of consent, (2) confirmed singleton pregnancy of fewer than 16 completed weeks of gestation at the time of consent, (3) planned to receive ongoing prenatal care in the Charleston, SC, area, and (4) the ability to provide written informed consent at the first visit. Exclusion criteria: Women with a pregnancy at greater than 16 weeks of gestation as calculated by their last menstrual period; pregnant women with preexisting calcium or parathyroid conditions or who required chronic diuretic or cardiac medication therapy, including calcium channel blockers, or who suffered chronic hypertension; pregnant women with active thyroid disease (eg, Graves disease, Hashimoto disease, or thyroiditis) also were excluded, but mothers on thyroid	G3: 26.6 ± 5.4 Gestational age at enrolment (wk), range G1: 7.1–18.4 G2: 8.4–17.6 G3: 6.4–21.4 Serum 25(OH)D (nmol/L), mean: G1: 61.6 ± 27.1 G2: 58.3 ± 22.3 G3: 58.2 ± 21.8 Assay: HPLC and RIA Weight status (%) BMI ≤30 kg/m^2: G1: 70.3 G2: 71.3 G3: 76.1 BMI >30 kg/m^2: G1: 29.7 G2: 28.7 G3: 23.9 Ethnicity (%): Black: G1: 25.2 G2: 30.3 G3: 28.2 Hispanic: G1: 40.5 G2: 39.3 G3: 37.6 White:	G2: vitamin D_3 1600 IU/d [40 μg/d] + vitamin D_3 400 IU/d [10 μg/d] G3: vitamin D_3 3600 IU/d [90 μg/d] + vitamin D_3 400 IU/d [10 μg/d] Background vitamin D intake (IU/d): G1: 181.6 ± 108.4 IU/d [4.5 ± 2.7 μg/d] G2: 195.8 ± 135.0 IU/d [4.9 ± 3.4 μg/d] G3: 204.2 ± 148.2 IU/d [5.1 ± 3.7 μg/d] Background calcium intake (mg/d): G1: 1063.6 ± 539.6 G2: 993.9 ± 514.0 G3: 1073.6 ± 491.9 Compliance Pill count method – vitamin D_3 (%) G1: 69 G2: 68 G3: 69 Serum 25(OH)D at 1 mo before delivery (nmol/L) G1: 79.4 ± 34.3	- no cut-off for elevated levels provided Urinary Ca/Cr ratio: - safety measure - collected at each visit (monthly study visits, which continued until delivery) - defined as Ca/Cr ratio of ≥0.8 mg/mg or ≥2.27 mmol/mmol - vitamin D_3 supplementation stopped if the U-Ca/Cr >1.0 mg/mg (>2.8 mol/mmol)	safety measure, including serumcalcium and urinary Ca/Cr. Moreover, review of adverse events by the Data safety and Monitoring Committee showed that not a single adverse event in this trial was attributed to vitamin D supplementation or circulating 25(OH)D levels. [It was reported that regarding the effect of S-25(OH)D concentrations on either blood calcium or urinary calcium level, no significant effects were observed, except for the relationship between low circulating 25(OH)D and U-Ca/Cr]	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	supplement with normal serologic parameters could participate in the study if they were without any other endocrine dysfunction. N participants, randomised*/completed/ analysed: G1: 164/111/111 G2: 166/122/122G3: 167/117/117 *received allocated intervention	G1: 34.2 G2: 30.3 G3: 34.2 Smoking status: NR Alcohol use: NR Health status: Healthy adults Season at study entry (%) April–September G1: 48.7 G2: 49.2 G3: 47.9 October–March G1: 51.4 G2: 50.8 G3: 52.1	G2: 105.4 ± 35.7 G3: 118.5 ± 34.9 Serum 25(OH)D at delivery (nmol/L) G1: 78.9 ± 36.5 G2: 98.3 ± 34.2 G3: 111.0 ± 40.4			
Roth et al. (2018) **MDIG** Bangladesh Latitude [assumed] 23.8° N from 17–24 wk of gestation to 26 wk postpartum Private funding	(RCT parallel) Inclusion criteria: Age 18 years and above; 17 to 24 completed weeks of gestation based on recalled last menstrual period and/or ultrasound; intends to reside in the trial catchment area for at least 18 months; provides written informed consent. Exclusion criteria: 1) History of any medical condition or medications that may predispose to vitamin D sensitivity, altered vitamin D	Sex: Females Age (y); median, range G1: 23, 18–38 G2: 22.5, 18–40 G3: 22, 18–35 G4: 22, 18–38 G5: 23, 18–38 Serum 25(OH)D (nmol/L) G1: 27.7 ± 13.8 G2: 27.4 ± 14.3 G3: 28.7 ± 14.0 G4: 27.0 ± 14.7 G5: 26.6 ± 13.2 Assay: HPLC–MS/MS (Information from the Supplementary Appendix)	Vitamin D_3 Doses G1: prenatal placebo + postpartum placebo + calcium 500 mg/d G2: prenatal vitamin D_3 4200 IU/wk [105 μg/wk = 15 μg/d] + postpartum placebo + calcium 500 mg/d G3: prenatal vitamin D_3 16 800 IU/wk [420 μg/wk = 60 μg/d] + postpartum placebo + calcium 500 mg/d G4: prenatal vitamin D_3 28 000 IU/wk [700 μg/	Hypercalcaemia: - for primary safety measure - maternal total serum calcium was measured at enrolment, 30 wk of gestation, delivery, 3 mo, and 6 mo postpartum, or during hospitalization (if feasible) - possible hypercalcaemia defined as any serum calcium >2.60 mmol/L (>10.4 mg/d) - confirmed hypercalcaemia defined as a serum calcium >2.60 mmol/L on a repeat specimen or a single serum calcium >2.80 mmol/L (>11.2 mg/dL).	Prenatal period: No episodes of confirmed hypercalcaemia. Possible hypercalcaemia, *n* of participants G1: 1 G2: 1 G3: 4 G4: 3 G5: 2 Postpartum period: Confirmed hypercalcaemia (asymptomatic), *n* of participants G1: 1 G2: 0	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	metabolism, and/or hypercalcaemia, including active tuberculosis or current therapy for tuberculosis, sarcoidosis, history of renal/ureteral stones, parathyroid disease, renal or liver failure, or current use of anti-convulsant; 2) High-risk pregnancy based on one or more of the following findings by point-of-care testing: Severe anemia (hemoglobin <70 g/L assessed by Hemocue); Moderate–severe proteinuria (≥300 mg/dl (3+ or 4+) based on urine dipstick; Hypertension (≥1 systolic blood pressure reading ≥140 mmHg and/or ≥1 diastolic blood pressure reading ≥ 90 mmHg, in repeat measurements taken at least one minute apart); 3) High-risk pregnancy based on one or more of the following findings by maternal history and/or ultrasound (Multiple gestation; Major congenital anomaly; Severe oligohydramnios);	BMI: NR Ethnicity [assumed]: Bangladeshi Smoking status: NR Alcohol use: NR Health status: Healthy pregnant women Season: Between March 2014 and Sept 2015	wk = 100 μg/d] + calcium 500 mg/d G5: prenatal vitamin D_3 28 000 IU/wk [700 μg/wk = 100 μg/d] + postpartum vitamin D_3 28 000 IU/wk [700 μg/wk = 100 μg/d] + calcium 500 mg/d Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method (%) Prenatal; 100% of scheduled doses: G1: 87.8 G2: 88.2 G3: 86.9 G4: 84.3 G5: 90.9 Postpartum; 100% of scheduled doses: G1: 76.9 G2: 70.9 G3: 74.7 G4: 67.8 G5: 74.5 Maternal serum 25(OH)D at delivery (nmol/L) G1: 24.30 ± 15.96		G3: 1 G4: 1 G5: 5 Possible hypercalcaemia, *n* of participants G1: 7 G2: 10 G3: 13 G4: 17 G5: 16	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	4) Unwillingness to stop taking non-study vitamin D or calcium supplements or a multivitamin containing calcium and/or vitamin D; 5) Currently prescribed vitamin D supplements as part of a physician's treatment plan for vitamin D deficiency; 6) Previous enrolment in the trial during a previous pregnancy. N participants, randomised/completed/ analysed: G1: 259/229/229 G2: 260/237/237 G3: 259/237/237 G4: 260/230/230 G5: 260/231/231 (Information on complete case analysis available in Table S6 in the Supplementary Appendix)		G2: 69.34 ± 19.44 G3: 100.37 ± 23.80 G4: 111.20 ± 27.83 G5: 113.49 ± 25.49 Maternal serum 25(OH)D at 3 mo postpartum (nmol/L) G1: 27.38 ± 12.70 G2: 31.72 ± 10.57 G3: 51.47 ± 11.69 G4: 59.97 ± 13.60 G5: 99.60 ± 20.60 Maternal serum 25(OH)D at 6 mo postpartum (nmol/L) G1: 29.87 ± 12.84 G2: 30.53 ± 11.26 G3: 45.01 ± 12.02 G4: 51.97 ± 12.83 G5: 103.83 ± 23.27			
Wagner et al. (2006) USA Latitude [assumed] 32.7° N	(RCT parallel) Inclusion criteria: Fully lactating mothers within 1 month postpartum planning to continue full breastfeeding for the next 6 mo.	Sex: Females Age (y) G1: 30.3 ± 3.3 G2: 28.3 ± 5.9 Serum 25(OH)D (nmol/L) G1: 80.5	Vitamin D_3 Doses G1: vitamin D_3 400 IU/d [10 µg/d] G2: vitamin D_3 6400 IU/d [160 µg/d]	Serum calcium and urinary Ca/Cr ratios: - safety measure - measured at baseline and then monthly - no cut-off for elevated levels provided	Maternal serum calcium and urinary Ca/Cr ratios remained in the normal range for both groups.	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
6 mo Public funding	Exclusion criteria: Included pre-existing type I or II diabetes, hypertension, parathyroid disease, and uncontrolled thyroid disease. N participants, randomised/completed/ analysed: All: 19/10/NR G1: 10/NR/NR G2: 9/NR/NR	G2: 85 Assay: HPLC and RIA techniques BMI (kg/m^2): NR Ethnicity (%) African American: G1: 11.1 G2: 11.1 White G1: 66.7 G2: 88.9 Hispanic: G1: 22.2 G2: 0 Smoking status: NR Alcohol use: NR Health status: Healthy adults Season: NR	Background vitamin D intake (IU/d) G1: 273.6 ± 274.5 IU/d [6.8 ± 6.9 µg/d] G2: 272.6 ± 114.5 [6.8 ± 2.9 µg/d] Background calcium intake (mg/d): G1: 1116.8 ± 587.3 G2: 1133.2 ± 286.7 Compliance Pill count method – vitamin D_3 (%) G1: ≥80 G2: ≥80 Serum 25(OH)D at 7 mo (nmol/L), mean G1: 38.4 ng/mL [96.0 nmol/L] G2: 58.8 ng/mL [147.0 nmol/L]			
General adult population						
Aloia et al. (2013) USA Latitude [assumed] 40.7° N 6 mo Private funding	RCT (parallel) Inclusion criteria: White, healthy, postmenopausal women. Exclusion criteria: Any chronic medical illness; osteoporosis; pregnancy; use of medications that influence bone metabolism or interfere with vitamin D metabolism; unexplained	Sex: Females Age (y) G1: 58.6 ± 6.7 G2: 60.0 ± 8.5 G3: 59.7 ± 7.1 G4: 57.6 ± 7.1 Serum 25(OH)D (nmol/L) G1: 67 ± 17 G2: 66 ± 19 G3: 64 ± 16 G4: 69 ± 17 Assay: RIA (Diasorin)	Vitamin D_3 Doses G1: double placebo G2: placebo + calcium 1200 mg/d G3: vitamin D_3 100 µg/d + placebo G4: vitamin D_3 100 µg/d + calcium 1200 mg/d Background vitamin D intake (IU/d)	Hypercalcaemia: - safety measure - serum calcium measured at baseline and at 15 and 28 weeks. - no cut-off provided Hypercalciuria: - safety measure - spot urine sample collected at baseline and at 15 and 28 weeks	**Hypercalcaemia** One incident of transient hypercalcaemia was noted in G4. **Hypercalciuria** Ca/Cr ratio >0.23: The frequency of values exceeding this concentration was highest in G4: 10 instances vs 1–2 in the other groups.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	weight loss during the previous year. N participants, randomised/completed/ analysed: G1: 31/24/31 G2: 35/26/35 G3: 47/36/47 G4: 46/34/46	BMI (kg/m^2) G1: 26.8 ± 3.9 G2: 26.7 ± 3.3 G3: 26.9 ± 3.6 G4: 27.4 ± 3.9 Ethnicity: White Smoking status: NR Alcohol use: NR Health status: Healthy population Season: winter	G1: 215 ± 205 IU/d [5.4 ± 5.1 µg/d] G2: 185 ± 140 IU/d [4.6 ± 3.5 µg/d] G3: 180 ± 163 IU/d [4.5 ± 4.1 µg/d] G4: 158 ± 105 IU/d [4.0 ± 2.6 µg/d] Background calcium intake (mg/d) G1: 890 ± 259 G2: 906 ± 320 G3: 876 ± 310 G4: 907 ± 288 Compliance Pill count method – vitamin D (%) All: 78% Pill count method – calcium (%) All: 78%	- defined as Ca/Cr ratio >0.23 and >0.37	Ca/Cr ratio >0.37: There was 1 episode of hypercalciuria in each group. No adverse events were believed to be related to supplementation other than hypercalciuria.	
Aloia et al. (2018) USA Latitude [assumed] 40.7° N 1 yr Unclear funding	RCT (parallel) Inclusion criteria: Serum 25(OH)D level < 32 ng/mL [80 nmol/L]; willingness to discontinue self-administration of vitamin D and calcium supplements; last menstrual period greater than five years	Sex: Females Age (y) G1: 62.2 (58.2–68.2) G2: 61.0 (57.5–67.9) Serum 25(OH)D (ng/mL) G1: 27.9 ± 5.7 ng/mL [69.8 ± 14.3 nmol/L] G2: 27.5 ± 7.3 ng/mL [68.8 ± 18.3 nmol/L] Assay: CLIA (DiaSorin)	Vitamin D_3 Doses [labeled] [1)] G1: vitamin D 600 IU/d [15 µg/d] + calcium 1200 mg/d G2: vitamin D_3 10 000 IU/d [250 µg/d] + calcium 1200 mg/d [1)] Assuming a dietary intake of 200 IU/d [5 µg/d] vitamin D and	Hypercalcaemia: - primary outcome - serum calcium measured at baseline and at 3, 6, 9, and 12 mo; non-corrected and albumin-corrected serum calcium - defined as serum calcium >10.2 mg/dL Hypercalciuria: - primary outcome	**Hypercalcaemia over 1 yr,** *n* of ≥1 event Serum calcium not corrected for albumin: G1: 11 G2: 15 Serum calcium corrected for albumin: G1: 11 G2: 14	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	ago; if present, hypertension and diabetes stable for the last three months. Exclusion criteria: T-score of the total hip below −2.5 standard deviations; history of osteoporotic fracture, hypercalciuria, hypercalcaemia or nephrolithiasis; hypercalciuria or hypercalcaemia at screening visit; use of medication that influences calcium or vitamin D metabolism; significant deviation from normal in medical history, physical examination or laboratory tests as evaluated by the primary investigator. N participants, randomised/completed/ analysed: G1: 66/45/66 G2: 66/47/66	BMI (kg/m^2) G1: 27.0 (23.6–30.8) G2: 28.0 (24.9–31.2) Ethnicity: White Smoking status: NR Alcohol use: NR Health status: Healthy population Season: NR	800 mg/d calcium in G1; assuming dietary intake of 800 mg/d calcium in G2. Analysed doses by corporation (Tischcon): G1: 710 IU [17.75 μg/d] G2: 12 069 IU [301.73 μg/d] Analysed doses by independent laboratory (Covance Laboratories) G1: 755 IU [18.88 μg/d] G2: 12 700 IU [317.5 μg/d] Background vitamin D intake: NR Background calcium intake (mg/d) G1: 900 (675–1214) G2: 878 (628–1114) Compliance Pill count method – vitamin D (%) G1: 87 G2: 85 Pill count method – calcium (%) G1: 84 G2: 81	- 24-h urine calcium collected at baseline and at 3, 6, 9, and 12 mo. - defined as 24-h urine calcium excretion >250 mg. Participants with hypercalcaemia or hypercalciuria first had their laboratories repeated. If repeated levels confirmed results, the calcium supplement was decreased to 600 mg/d. If laboratory values were still high at the next follow-up visit, the calcium supplement was discontinued. If the levels on the subsequent follow-up visit still showed hypercalciuria or hypercalcaemia, the vitamin D supplement was discontinued.	In G2, 23% of patients had hypercalcaemia at least once during the study period (15 patients had 23 events) vs 17% (11 patients had 17 events) in G1. After correcting serum calcium for albumin, 21% of patients in G2 had hypercalcaemia at least once during the study duration (14 patients had 20 events) vs 17% (11 patients had 17 events) in G1. **Hypercalcaemia over 1 yr,** OR (95% CI) Serum calcium not corrected for albumin: G2 vs G1: 0.84 (0.25, 2.79) Serum calcium corrected for albumin: G2 vs G1: 0.74 (0.22, 2.4) **Hypercalciuria over 1 yr,** *n* of ≥1 event G1: 19 G2: 34	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
			Serum 25(OH)D (ng/mL) at 1 year: G1: 33.7 ± 6.1 ng/mL [84.3 ± 15.3 nmol/L] G2: 86.6 ± 26.4 ng/mL [215.5 ± 66.0 nmol/L] Calcium intake at 1 year (mg/d): G1: 1843 (1728–1986) G2: 1986 (1779–2136)		In G2, of the 34 subjects with an instance of hypercalciuria, 14 developed hypercalciuria once, 15 developed hypercalciuria 2–3 times, and 5 subjects developed hypercalciuria 4 times during the study. In G1, of the 19 subjects with an instance of hypercalciuria, 10 were hypercalciuric once, 7 were hypercalciuric 2–3 times and 2 were hypercalciuric 4 times during the study. **Hypercalciuria over 1 yr,** OR (95% CI) G2 vs G1: 3.6 (1.39, 9.30) [the OR does not refer to recurrent cases alone]	
Billington et al. (2020) Canada Latitude [assumed] 51.0° N 3 y Private funding	RCT (parallel) Inclusion criteria: Healthy men and postmenopausal women; 55–70 years; lumbar spine and total hip bone mineral density T scores greater than −2.5, assessed using dual–x-ray absorptiometry. Exclusion criteria:	Sex (% Females) G1: 48.4 G2: 53.6 G3: 50.8 Age (y) G1: 62.0 ± 4.2 G2: 62.7 ± 4.3 G3: 62.0 ± 4.1 Serum 25(OH)D (nmol/L) G1: 76 ± 21 G2: 80 ± 20	Vitamin D_3 Doses G1: vitamin D_3 400 IU/d [10 μg/d] G2: vitamin D_3 4000 IU/d [100 μg/d] G3: vitamin D_3 10 000 IU/d [250 μg/d] If dietary calcium intake was <1200 mg/day, a daily supplement	Hypercalcaemia: - secondary outcome - serum calcium measured at baseline and at 3, 6, 12, 18, 24, 30, and 36 mo - defined as total serum calcium >2.55 mmol/L, - participants were asked to discontinue the study intervention if repeat testing demonstrated persistent hypercalcaemia.	**Hypercalcaemia** Hypercalcaemia, *n* of events G1: 0 G2: 4 G3: 12 *P* = 0.02 for trend. Hypercalcaemia, *n* of ≥1 event G1: 0 G2: 4	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	Serum 25(OH)D $<$ 30 or $>$ 125 nmol/L; serum calcium $>$2.5 or $<$2.10 mmol/L; consumption of vitamin D supplements of more than 2000 IU/day within the previous 6 mo; use of bone-active medication within the past 2 y; disorders known to affect vitamin D metabolism. N participants, randomised/completed/analysed: G1: 124/113/124 G2: 125/115/125 G3: 124/109/124	G3: 78 $\pm$ 18 Assay: CLIA (Diasorin Liaison XL system) BMI (kg/m^2) G1: 27.7 $\pm$ 4.4 G2: 27.8 $\pm$ 5.0 G3: 27.2 $\pm$ 4.4 Ethnicity [assumed]: Caucasian Current smokers (%) G1: 2.4 G2: 1.6 G3: 4.0 Alcohol use: NR Health status: Healthy population Season: NR	containing either 300 mg or 600 mg elemental calcium was provided to approximate a total daily intake of 1200 mg. Participants starting calcium supplementation at baseline (%) G1: 75 G2: 63.2 G3: 73.4 During the study, 32.7% of supplement takers discontinued taking the supplement and 18.2% of those not taking supplement initiated the supplementation. Background vitamin D intake G1: 166 $\pm$ 88 IU/d [4.2 $\pm$ 2.2 µg/d] G2: 178 $\pm$ 92 IU/d [4.5 $\pm$ 2.3 µg/d] G3: 188 $\pm$ 120 IU/d [4.7 $\pm$ 3.0 µg/d] Background calcium intake (mg/d) G1: 600 $\pm$ 303 G2: 624 $\pm$ 279 G3: 639 $\pm$ 344	Hypercalciuria: - secondary outcome - 24-h urine collected at baseline and at 12, 24, and 36 mo - spot urine collected for Ca/Cr ratio at 6, 18, and 30 mo, and at other time points in cases for which the participant could not provide a 24-h urine sample or required follow-up testing after hypercalciuria was detected - defined as 24-h urine calcium excretion $>$7.5 mmol/day for participants of $<$75 kg body weight; a weight-based cutoff $<$0.1 mmol/kg/day was used for those $>$75 kg - Ca/Cr ratio was served as a safety flag for the identification of significant hypercalciuria; a ratio of $\geq$1.0 mmol/mmol at 6, 18, or 30 mo and at a follow-up review of the participant's next 24-h urine calcium excretion resulted in discontinuation of the study treatment. Participants were asked to discontinue the study	G3: 11 16 episodes of mild hypercalcaemia (serum calcium 2.56–2.64 mmol/L) occurred in 15 participants. Hypercalcaemia resolved on follow-up testing in all cases. Calcium intake was reduced prior to follow-up testing in 10 of these cases (discontinued in 8 and decreased in 2). 2 participants in G3 withdrew from the study due to hypercalcaemia; a diagnosis of primary hyperparathyroidism was suspected in 1. 12 events occurred within the first 12 mo and the remaining 4 events occurred at month 30. 1 participant in G3 experienced 2 episodes of transient hypercalcaemia, at mo 6 and mo 30. **Hypercalciuria** Hypercalciuria, *n* of events G1: 27	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
			Compliance Adherence rate of vitamin D doses (%) G1: 99.6 G2: 99.7 G3: 99.1 Serum 25(OH)D at 3 mo (nmol/L) G1: 76 ± 17 G2: 114 ± 22 G3: 187 ± 38	intervention if repeat testing demonstrated persistent hypercalcaemia.	G2: 40 G3: 56 Hypercalciuria, *n* of ≥1 event G1: 21 G2: 28 G3: 38 Recurrent episodes of elevated 24-h urine calcium excretion were common, occurring in 5 (4.0%), 8 (6.4%), and 14 (11.3%) participants in G1, G2, and G3, respectively. No participants discontinued the study treatment because of hypercalciuria.	
Brohult et al. (1973) Sweden Latitude [assumed] 60.1° N 1 yr Private funding	CT Inclusion criteria: Out-patients with definite rheumatoid arthritis. Exclusion criteria: Treatment with steroid, gold, or antimalaria therapy (the institution of such therapy was avoided as far as possible). N participants, randomised/completed/analysed: G1: 25/21/NR	Sex (% Females) 68 Age (y); mean, range All: 52, 18–69 G1: 53, NR G2: 51, NR Serum 25(OH)D: NR BMI: NR Ethnicity [assumed]: Caucasian Smoking status: NR Alcohol use: NR	Vitamin D_2 Doses G1: placebo G2: vitamin D_2 100 000 IU/d [2500 μg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance: NR	Serum calcium - secondary outcome - serum calcium checked every other month - no cut-off for elevated levels provided	Serum calcium did not change significantly in either of the groups. One of the patients G2 had a serum calcium value of **7.0** mg/l [3.5 mmol/L] after 10 months' treatment; this was the only serum calcium value to exceed the normal upper limit.	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	G2: 24/18/NR	Health status: Rheumatoid arthritis Season: NR				
Burnett-Bowie et al. (2012) USA Latitude [assumed] 42.3° N 12 wk Mixed funding	RCT (parallel) Inclusion criteria: Healthy people aged 18–45 y; serum 25OHD level ≤ 20 ng/ml [50 nmol/L]; normal kidney, liver, and thyroid function; males had normal testosterone levels; females had regular menses (oral contraceptive use was allowed). Exclusion criteria: disorders or use of medications known to affect phosphate or vitamin D metabolism; histories of nephrolithiasis, diabetes mellitus, malabsorption, recent ethanol abuse, or clinically significant disease N participants, randomised/completed/analysed: All: 92/90/90	Sex (% Females) G1: 60 G2: 62 Age (y) G1: 27 (22–39) G2: 26 (23–32) Serum 25(OH)D (ng/mL) G1: 18 ± 7 ng/mL [45 ± 17.5 nmol/L] G2: 18 ± 7 ng/mL [45 ± 17.5 nmol/L] Assay: LC–MS/MS BMI (kg/m^2) G1: 26 ± 7 G2: 25 ± 4 Ethnicity (%) White/Caucasian: G1: 40 G2: 45 Black/African American: G1: 36 G2: 20 Asian: G1: 12 G2: 7.5 Multiple races/other:	Vitamin D_2 Doses G1: placebo G2: vitamin D_2 50 000 IU/wk [1250 μg/wk = 178.6 μg/d] Daily calcium intake was maintained at 1000–1500 mg/d in both groups through diet and/or supplements. Background vitamin D intake (IU/d) G1: 65 (31–130) IU/d [1.6 (0.8–3.3) μg/d] G2: 150 (75–231) IU/d [3.8 (1.9–5.8) μg/d] Background calcium intake (mg/d) G1: 939 ± 523 G2: 1162 ± 651 Compliance Taking 85% of the study pills (*n*)	Hypercalcaemia: - secondary outcome - serum calcium measured at baseline and at 4, 8, and 12 wk - no cut-off provided - participants were monitored for hypercalcaemia at each visit and withdrawn if necessary.	No subject developed hypercalcaemia.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	G1: NR/50/50 G2: NR/40/40	G1: 12 G2: 7.5 Smoking status: NR Alcohol use: NR Health status: Healthy population, vitamin D-deficient Season at baseline (%) Fall: G1: 28 G2: 37.5 Spring: G1: 24 G2: 20 Summer: G1: 30 G2: 25 Winter: G1: 14 G2: 22.5	G1: 3 G2: 2 The remaining participants were 100% compliant. Serum 25(OH)D at wk 12 (ng/mL): G1: No significant change from baseline) G2: 43 ± 12 ng/mL [107.5 ± 30 nmol/L]			
Ceglia et al. (2013) USA Latitude [assumed] 42.4° N 4 mo Private funding	RCT (parallel) Inclusion criteria: Ambulatory; community-dwelling; postmenopausal women; ≥65 y; maintaining usual level of physical activity and habitual diet during the study to limit the impact of physical activity and dietary variation on skeletal muscle; moderate risk for disability based on	Sex: Females Age (y) G1: 80 ± 5 G2: 76 ± 4 Serum 25(OH)D (nmol/L) G1: 48.3 ± 8.8 G2: 43.6 ± 10.3 Assay: RIA (Diasorin) BMI (kg/m^2) G1: 25 ± 3 G2: 29 ± 7 Ethnicity (%)	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 4000 IU/d [100 μg/d] Background vitamin D intake: NR Background calcium intake (mg/d) G1: 1316 ± 1257 G2: 963 ± 663	Elevated urine Ca/Cr ratio: - safety measure - spot urine sample for Ca/Cr ratio collected at baseline, on day 30, and at 4 mo - cut-off defined as >0.325 (corresponding to a 24-h urine calcium of 350 mg) - if elevated Ca/Cr ratio occurred, the study pills were discontinued, and a repeat spot urine and serum calcium level were drawn within the following 7 days; if the ratio	One subject in G2 had a transient high spot urine Ca/Cr ratio, which resolved on follow-up testing on study pills.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
	a short physical performance battery score of ≤9 (out of a possible 12 points) Exclusion criteria: Active parathyroid disease; chronic kidney disease; nephrolithiasis; malignancy; liver disease; malabsorption; diabetes; unstable heart disease; severe osteoarthritis; neurodegenerative disease; vitamin D intake >400 IU/d or a 25OHD level < 22.5 or > 60 nmol/L; a calcium intake >1000 mg/d; abnormal serum calcium or 24-h urinary calcium >275 mg; medications such as hormone replacement therapy in the last 6 months, oral glucocorticoids in the last month, diuretics, antiseizure medications, drugs to treat osteoporosis in the last year, and prescribed antiplatelet and anticoagulant medications; travel to latitudes below 35° N; use of tanning beds, wheelchair, walker, and nasal oxygen	Caucasian G1: 92 G2: 78 Smoking status: NR Alcohol use: NR Health status: Older mobility-limited women Season: NR	Compliance Pill count method: Was performed but results NR; stated that adherence to the intervention was high. Serum 25(OH)D at 4 mo (nmol/L) G1: 52.5 ± 17.1 G2: 80.0 ± 11.5	normalised and serum calcium was normal, pills could be resumed, but repeat testing on pills was performed during the following 2 and 4 weeks.		

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	N participants, randomised/completed/ analysed: G1: 13/12/12 G2: 11/9/9					
Diamond et al. (2013) Australia Latitude [assumed] 33.9° S 3 mo Unclear funding	RCT (parallel) Inclusion criteria: serum 25(OH) D $\leq$ 50 nmol/L; no evidence of hypocalcaemia (serum calcium <2.15 mmol/L), significant renal impairment (serum creatinine >0.15 mmol/L), malignancy or were receiving treatment with vitamin D_3, calcitriol or high-dose oral calcium supplements (>1,200 mg/ day of elemental calcium) for at least 6 mo prior to the study. Exclusion criteria: NR N participants, randomised/completed/ analysed: All: 30/26/26 G1: NR/11/11 G2: NR/15/15	Sex (% Females) G1: 72.7 G2: 40.0 Age (y) G1: 45.5 $\pm$ 16.9 G2: 47.4 $\pm$ 14.4 Serum 25(OH)D (nmol/L) G1: 41.2 $\pm$ 11.9 nmol/L G2: 35.8 $\pm$ 10 nmol/L Assay: CLIA (DiaSorin LIAISON) BMI: NR Ethnicity [assumed]: Caucasian Smoking status: NR Alcohol use: NR Health status: Vitamin D deficient patients Season: Recruitment in Feb to Apr (autumn)	Vitamin D_3 Doses G1: vitamin D_3 2000 IU/d [50 µg/d] G2: vitamin D_3 5000 IU/d [125 µg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method: NR Serum 25(OH)D at 3 mo (nmol/L): G1: 75.3 $\pm$ 15.9 G2: 114.4 $\pm$ 22.2	Hypercalcaemia: - secondary outcome - serum calcium measured at baseline and at 1, 2 and 3 mo - no cut-off provided	No patient demonstrated hypercalcaemia.	**3**
Drincic et al. (2013) USA	RCT (parallel) Inclusion criteria: BMI $\geq$30 kg/m^2	Sex (% Females) G1: 59 G2: 55 G3: 65	Vitamin D_3 Doses (labeled) G1: vitamin D_3 1000 IU/d [25 µg/d]	Hypercalcaemia: - safety measure - serum calcium measured at baseline and at 1, 3, 6, 10 and 21 wk	There was no increase in serum calcium levels during the study in any treatment group; there	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Latitude 41.2° N 21 wk Private funding	Exclusion criteria: Past or current hepatic or kidney disease; taking medications that affect vitamin D metabolism; any malabsorptive conditions from medical or surgical causes; history of hypercalcaemia, sarcoidosis or active kidney stones; history of fractures; current use of bisphosphonates; no more than 800 IU [20 μg] daily of vitamin D from food and supplements; outdoor job during the previous summer; plans to visit a sunny region during the study. N participants, randomised/completed/analysed: All: 67/58/62 G1: NR/NR/22 G2: NR/NR/20 G3: NR/NR/20	Age (y) G1: 47.1 ± 12.5 G2: 45.7 ± 12.6 G3: 44.5 ± 12.9 Serum 25(OH)D (ng/mL) G1: 20.3 ± 6.4 [50.8 ± 16 nmol/L] G2: 26.5 ± 6.7 [66.3 ± 16.8 nmol/L] G3: 23.2 ± 15.2 [58 ± 38 nmol/L] Assay: RIA (DiaSorin) BMI (kg/m^2) G1: 36.7 ± 4.6 G2: 36.1 ± 5.1 G3: 37.9 ± 7.2 Ethnicity: Caucasian Smoking status: NR Alcohol use: NR Health status: Healthy women and men with BMI ≥30.0 kg/m2 Season: Winter	G2: vitamin D_3 5000 IU/d [125 μg/d] G3: vitamin D_3 10 000 IU/d [250 μg/d] Analysed vitamin D_3 doses G1: 911 IU [22.8 μg/d] G2: 5747 IU [143.7 μg/d] G3: 11 495 IU [287.4 μg/d] Background vitamin D intake (IU/d) G1: 207 ± 277 [5.2 ± 6.9 μg/d] G2: 203 ± 230 [5.1 ± 5.8 μg/d] G3: 279 ± 313 [7.0 ± 7.8 μg/d] Background calcium intake: NR Compliance Pill count method – vitamin D (%) All: 95 on average, range 94–97. Serum 25(OH)D change at 21 weeks (ng/mL) G1: 12.4 ± 9.7 [31 ± 24.3 nmol/L] G2: 27.8 ± 10.2 [69.5 ± 25.5 nmol/L]	- no cut-off provided	were no hypercalcaemia events during the study.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
			G3: 50.7 ± 16.4 [126.8 ± 41 nmol/L]			
Gallagher et al. (2012); Gallagher et al. (2014a) **ViDOS** USA Latitude [assumed] 41.2° N 1 yr Mixed funding 2012 for baseline info, 2014 for the outcomes	RCT (parallel) Inclusion criteria: Healthy, white, postmenopausal women aged 57 to 90 years who were at least 7 years postmenopausal (determined from the history of their last menstrual period) with vitamin D insufficiency Exclusion criteria: Significant health problems,active nephrolithiasis or history of more than two kidney stones in their lifetime, chronic renal failure (serum creatinine > 1.4 mg/dL), chronic liver disease, medical conditions severe enough to prevent reasonable physical activity, serum 25(OH)D levels < 5 ng/mL (12.5 nmol/L), serum calcium levels of 10.3 mg/dL (2.575 mmol/L) or more or serum calcium levels more than 0.3 mg/dL higher than the upper normal limit on two	Sex: Females Age (y) G1: 66 ± 6.5 G2: 68 ± 8.6 G3: 68 ± 8.1 G4: 66 ± 7.4 G5: 66 ± 6.3 G6: 69 ± 7.7 G7: 66 ± 7.1 G8: 65 ± 6.1 Serum 25(OH)D (nmol/L) G1: 37.7 ± 9.1 G2: 37.8 ± 10.8 G3: 39.0 ± 9.5 G4: 37.4 ± 10.2 G5: 38.2 ± 10.1 G6: 39.8 ± 8.2 G7: 37.2 ± 9.2 G8: 38.6 ± 9.1 Assay: RIA (Diasorin) BMI (kg/m^2) G1: 31.1 ± 5.3 G2: 30.3 ± 5.4 G3: 28.2 ± 6.1 G4: 30.0 ± 5.4 G5: 30.4 ± 5.4 G6: 30.2 ± 5.7 G7: 29.7 ± 6.4 G8: 32.1 ± 6.2 Ethnicity: White Smoking status (%)	Vitamin D_3 Doses (labeled) G1: placebo + calcium[2)] G2: vitamin D_3 400 IU/d [10 µg/d] + calcium[2)] G3: vitamin D_3 800 IU/d [20 µg/d] + calcium[2)] G4: vitamin D_3 1600 IU/d [40 µg/d] + calcium[2)] G5: vitamin D_3 2400 IU/d [60 µg/d] + calcium[2)] G6: vitamin D_3 3200 IU/d [80 µg/d] + calcium[2)] G7: vitamin D_3 4000 IU/d [100 µg/d] + calcium[2)] G8: vitamin D_3 4800 IU/d [120 µg/d] + calcium[2)] [2)] Calcium to maintain total intake between 1200 to 1400 mg/d (was based on a baseline 7-day food diary) Analysed vitamin D_3 doses (mean of the capsules measured every 6 mo over 3 y) G2: 503 IU [12.6 µg] G3: 910 IU [22.8 µg] G4: 1532 IU [38.3 µg] G5: 2592 IU [64.8 µg] G6: 2947 IU [73.8 µg] G7: 4209 IU [105.2 µg]	Hypercalcaemia: - safety measure - serum calcium measured at baseline and at 3, 6, 9, and 12 mo - during the study, defined as serum calcium >0.3 mg/dL [0.075 mmol/L] above the upper limit of reference; i.e. >10.5 mg/dL [2.625 mmol/L] - for poststudy analysis, defined as serum calcium level ≥ 10.3 mg/dL [2.75 mmol/L]; i.e. exceeding the upper reference limit Hypercalciuria: - safety measure - 24-h urine calcium measured at baseline and at 3, 6, 9, and 12 months. - during the study, defined as a 24-h urine calcium >400 mg [10 mmol/d] at any study visit - for poststudy analysis, defined as a 24-h urine calcium >300 mg [7.5 mmol] - for poststudy analysis, severe hypercalciuria defined as a 24-h urine calcium >400 mg [10 mmol]	**Hypercalcaemia** Serum calcium ≥2.75 mmol/L, *n* of events G1: 3 G2: 2 G3: 1 G4: 5 G5: 6 G6: 1 G7: 1 G8: 1 Serum calcium ≥2.75 mmol/L, *n* of ≥1 event G1: 1 G2: 1 G3: 1 G4: 4 G5: 4 G6: 1 G7: 1 G8: 1 Hypercalcaemias were transient and did not lead to reduction of calcium or vitamin D doses. **Hypercalciuria** 24-h urine calcium >7.5 mmol/d, *n* of events G1: 8	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	baseline tests, and 24-h urine calcium levels > 300 mg/dL (7.5 mmol) on two baseline tests; taking currently bisphosphonates or had taken them for > 3 months in the past were excluded; use of fluoride, parathyroid hormone (PTH) or its derivatives, calcitonin, estrogen (in the last 6 mo), corticosteroids (> 10 mg/d), phenytoin or phenobarbital, or high-dose thiazide (> 37.5 mg/d); multivitamins containing vitamin D were not allowed in the study. N participants, randomised/completed/analysed: G1: 21/18/19 G2: 20/16/18 G3: 21/17/18 G4: 20/19/19 G5: 21/20/20 G6: 20/18/18 G7: 20/16/17 G7: 20/18/18	Current: G1: 19 G2: 10 G3: 5 G4: 20 G5: 5 G6: 15 G7: 0 G8: 10 Former: G1: 33 G2: 35 G3: 33 G4: 40 G5: 38 G6: 20 G7: 50 G8: 45 Never: G1: 48 G2: 55 G3: 62 G4: 40 G5: 57 G6: 65 G7: 50 G8: 45 Alcohol users (%) G1: 33 G2: 35 G3: 57	G8: 4937 IU [123.4 μg] Background vitamin D intake (IU/d) G1: 105 ± 61 IU/d [2.6 ± 1.5 μg/d] G2: 98 ± 58 IU/d [2.5 ± 1.5 μg/d] G3: 135 ± 70 IU/d [3.4 ± 1.8 μg/d] G4: 125 ± 71 IU/d [3.1 ± 1.8 μg/d] G5: 98 ± 55 IU/d [2.5 ± 1.4 μg/d] G6: 109 ± 62 IU/d [2.7 ± 1.6 μg/d] G7: 106 ± 83 IU/d [2.7 ± 2.1 μg/d] G8: 137 ± 86 IU/d [3.4 ± 2.2 μg/d] Background calcium intake (mg/d) G1: 593 ± 182 G2: 606 ± 212 G3: 741 ± 247 G4: 754 ± 244 G5: 612 ± 190 G6: 725 ± 263 G7: 673 ± 324 G8: 768 ± 348	If hypercalcaemia or hypercalciuria developed during the treatment period, calcium measurements were repeated within 2 weeks; if hypercalcaemia or hypercalciuria persisted, calcium supplements were withdrawn, dietary calcium was rechecked, and a repeat calcium measurement was performed within 2 weeks; if elevation persisted, vitamin D was withdrawn. Overcollection of 24-h urine was considered based on an individual's mean 24-h urine creatinine level being 20% higher than the mean for all tests.	G2: 13 G3: 11 G4: 8 G5: 15 G6: 10 G7: 10 G8: 6 24-h urine calcium >7.5 mmol/d, *n* of ≥1 event G1: 4 G2: 8 G3: 7 G4: 5 G5: 6 G6: 6 G7: 6 G8: 5 24-h urine calcium >10 mmol/d, *n* of ≥1 event G1: 3 G2: 2 G3: 3 G4: 2 G5: 4 G6: 1 G7: 2 G8: 2 24-h urine calcium levels >10 mmol/d were	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
		G4: 50 G5: 52 G6: 70 G7: 70 G8: 80 Health status: Healthy. Season: NR	Compliance Pill count method – vitamin D_3 capsules (%) All: 94 on average Pill count method – calcium tablets (%) All: 91 on average Serum 25(OH)D at 1 yr: G1 vs. the other groups: levels were significantly lower (visual presentation)		normalised at repeated testing in all but 5 participants. Calcium supplements were withdrawn in 2 of these participants and their follow-up urine calcium was <7.5 mmol/L. Hypercalciuria still continued in 2 participants, and both calcium and vitamin D_3 were discontinued permanently. One participant refused follow-up testing. The respective groups were not reported. One participant with 24-h urine calcium >7.5 mmol/d was not included in the results above due to overcollection of 24-h urine. [additional data on recurrent hypercalciuria cases were received from the authors upon request]	
Gallagher et al. (2013) **ViDOS** USA	RCT (parallel) Inclusion criteria: Postmenopausal African American women aged 57–90 years; vitamin D	Sex: Females Age (y) G1: 66.6 ± 6.9 G2: NR G3: 69.3 ± 8.9	Vitamin D_3 Doses (labeled) G1: placebo G2: vitamin D_3 400 IU/d [10 μg/d] + calcium[3,4)]	Hypercalcaemia: - safety measure - serum calcium measured at baseline and at 12 mo	**Hypercalcaemia** Hypercalcaemia occurred in 7% of women. Stated there was no correlation	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Latitude [assumed] 41.2° N and 39.7° N 1 year Public funding	insufficiency with serum 25 (OH)D of <20 ng/mL [50 nmol/L]. Exclusion criteria: Significant comorbidities; history of cancer except skin cancer within last 10 years; terminal illness; previous hip fracture; hemiplegia; uncontrolled type 1 diabetes ± significant proteinuria or fasting blood glucose greater than 140 mg in type 2 diabetes; active kidney stone disease or kidney stones >2 times in lifetime; chronic renal failure (serum creatinine >1.4 mg/dL); evidence of chronic liver disease including alcoholism; physical conditions like rheumatoid arthritis, osteoarthritis, and heart failure severe enough to prevent reasonable physical activity; having severe vitamin D deficiency; serum 25OHD <5 ng/mL (<12.5 nmol/L) or > 20 ng/mL (>50 nmol/L); BMI > 45 kg/m2; serum	G4: 67.1 ± 6.2 G5: 64.0 ± 6.5 G6: NR G7: NR G8: 65.6 ± 6.5 Serum 25(OH)D (ng/mL) G1: 13.6 ± 3.8 [34 ± 9.5 nmol/L] G2: NR G3: 13.5 ± 4.6 [33.8 ± 11.5 nmol/L] G4: 12.5 ± 4.7 [31.3 ± 11.8 nmol/L] G5: 13.8 ± 4.0 [34.5 ± 10 nmol/L] G6: NR G7: NR G8: 13.6 ± 4.7 [34 ± 11.8 nmol/L] Assay: RIA (DiaSorin) BMI (kg/m2): G1: 31.4 ± 6.1 G2: NR G3: 32.1 ± 5.4 G4: 32.3 ± 9.6 G5: 35.0 ± 7.4 G6: NR G7: NR G8: 33.3 ± 5.5 Ethnicity: African American Smoking status: NR Alcohol use: NR	G3: vitamin D_3 800 IU/d [20 μg/d] + calcium[3)] G4: vitamin D_3 1600 IU/d [40 μg/d] + calcium[3)] G5: vitamin D_3 2400 IU/d [60 μg/d] + calcium[3)] G6: vitamin D_3 3200 IU/d [80 μg/d] + calcium[3)] G7: vitamin D_3 4000 IU/d [100 μg/d] + calcium[3,4)] G8: vitamin D_3 4800 IU/d [120 μg/d] + calcium[3,4)] [3)] Calcium to maintain a total intake of 1200–1400 mg/d. [4)] The group was excluded from the analyses due to the small number of participants. Analysed vitamin D_3 doses (mean of the capsules measured every 6 mo over 3 y) G2: 503 IU [12.6 μg] G3: 910 IU [22.8 μg] G4: 1532 IU [38.3 μg] G5: 2592 IU [64.8 μg] G6: 2947 IU [73.8 μg] G7: 4209 IU [105.2 μg] G8: 4937 IU [123.4 μg] Background vitamin D intake: NR	- defined as >0.3 mg/dl above the upper limit of the normal range, which was >10.5 mg/dL in Omaha and > 10.8 mg/dL in Indiana at any visit. "Fasting blood samples were collected at all visits (baseline, 3, 6, 9, and 12 mo. . . . . .comprehensive panel including serum calcium, creatinine, complete blood count, and lipid profile was performed at baseline and 12 mo". . ." A basic metabolic panel was done at 3, 6, and 9 mo" Hypercalciuria: - safety measure - 24-h urine calcium measured at baseline and at 3, 6, 9, and 12 mo - during the study defined as 24-h urine calcium >400 mg (10 mmol) at any of the follow-up visits (based on the limit established for Caucasians). - in a post hoc analysis, a upper limit of 270 mg was defined for African Americans If hypercalcaemia or hypercalciuria developed during the treatment period,	between vitamin D dose and hypercalcaemia. One participant in G1 discontinued intervention due to hypercalcaemia. (Figure 1) **Hypercalciuria** 24-h urine calcium >270 mg: Incidence was 15% 24-h urine calcium >300 mg: Incidence was 9% based on the limit of 300 mg. Stated there was no correlation between the vitamin D dose and hypercalciuria.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
	calcium <10.3 mg/dL (>2.57 mmol/L) or 0.3 mg/dL (>0.075 mmol/L) more than the upper limit of normal on 2 baseline tests; 24-h urine calcium >290 mg/dL (7.25 mmol) on 2 baseline tests; bone mineral density T-score less than −3 on spine or hip specific to race; on bisphosphonates for >3 months in the past; had been taking fluoride, PTH, or derivatives, eg, teriparatide in the last 6 months; had previous treatment within the last 6 months with calcitonin or estrogen, chronic high-dose corticosteroid therapy (>10 mg/d) for more than 6 months; were currently on anticonvulsants (phenytoin, phenobarbital), high-dose thiazide therapy (>37.5 mg/d), and any drugs interfering with vitamin D metabolism; or the subjects were not able to give informed consent. N participants, randomised/completed/analysed:	Health status: Healthy Season: NR	Background calcium intake (mg/d) G1: 540 ± 190 G2: NR G3: 604 ± 282 G4: 523 ± 197 G5: 529 ± 231 G6: NR G7: NR G8: 584 ± 210 Compliance Pill count method – vitamin D (%) in Omaha: 91 in Indiana: 81 Pill count method – calcium (%) in Omaha: 79 in Indiana: 70 Serum 25(OH)D at 1 yr G1 vs other groups: significantly lower (visual presentation)	calcium measurements were repeated within 2 weeks; if hypercalcaemia or hypercalciuria persisted, calcium supplements were withdrawn, dietary calcium was rechecked, and a repeat calcium measurement was performed within 2 weeks; if elevation persisted, vitamin D was withdrawn.		

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	G1: 17/14/14 G2: 2/2/0 G3: 24/20/20 G4: 23/18/18 G5: 23/20/20 G6: 3/3/0 G7: 4/4/0 G8: 14/10/10					
Grimnes et al. (2012) Norway Latitude 69° N 1 yr Private funding	RCT (parallel) Inclusion criteria: Postmenopausal women; aged 50–80 y; a T-score in total hip or lumbar spine (L2–4) ≤ -2.0 Exclusion criteria: Hormone replacement therapy or other therapy affecting bone remodeling during the last 12 months before enrolment; use of steroids; renal stone disease; systolic blood pressure $>$ 175 mmHg or diastolic blood pressure $>$ 105 mmHg; serum creatinine $>$110 μmol/l, suspected primary hyperparathyroidism (serum calcium $>$2.55 mmol/L; serum calcium $>$2.50 mmol/L combined with plasma PTH $>$5.0 pmol/l; serum	Sex: Females Age (y) G1: 63.5 $\pm$ 6.8 G2: 62.9 $\pm$ 7.6 Serum 25(OH)D (nmol/L) G1: 71.2 $\pm$ 22.3 G2: 70.7 $\pm$ 23.0 Assay: LC–MS/MS BMI (kg/m^2) G1: 24.6 $\pm$ 3.2 G2: 25.0 $\pm$ 3.4 Ethnicity [assumed]: Caucasian Smoking status (%) Never smoked: G1: 24 G2: 23 Former smoker: G1: 39 G2: 40 Current smoker: G1: 38 G2: 37 Alcohol use: NR	Vitamin D_3 Doses G1: vitamin D_3 800 IU/d [20 μg/d] + calcium 500 mg G2: vitamin D_3 6500 IU/d [162.5 μg/d] + calcium 500 mg Background vitamin D intake (μg/d) G1: 8.1 $\pm$ 6.0 G2: 9.1 $\pm$ 6.2 Background calcium intake (mg/d) G1: 1044 $\pm$ 552 G2: 1062 $\pm$ 524 Compliance Pill count method – vitamin D (%) All: 97 Pill count method – calcium (%) All: 92	Hypercalcaemia: - safety measure - serum calcium measured at baseline and 3, 6, 9 and 12 mo - defined as serum calcium $\geq$2.56 mmol/L - modest hypercalcaemia defined as 2.60–2.80 mmol/L - severe hypercalcaemia defined as $>$2.80 mmol/L - according to the study protocol, participants experiencing severe hypercalcaemia should be excluded.	Serum calcium 2.56–2.59 mmol/L during the study, *n* of participants G1: 8 G2: 9 Serum calcium $\geq$2.60 mmol/L during the study, *n* of participants G1: 4 G2: 9 No severe hypercalcaemias occurred. The modest hypercalcaemias (serum calcium 2.60–2.80 mmol/L) occurred at serum 25(OH)D levels across a range of 64–256 nmol/L. These participants continued in the study, and all the hypercalcaemias had resolved at retesting without stopping the treatment.	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	calcium >2.45 mmol/L combined with plasma PTH >7.0 pmol/l); chronic disease like ischemic heart disease, diabetes, granulomatous disease, and cancer N participants, randomised/completed/ analysed: G1: 149/135/135 G2: 148/140/140	Health status (%) Asthma/COPD: G1: 10 G2: 6 Thyroid disease: G1: 9 G2: 13 Arthrosis: G1: 9 G2: 11 Other musculoskeletal disease: G1: 11 G2: 15 Hypertension: G1: 19 G2: 17 Hypercholesterolemia: G1: 11 G2: 12 Previous osteoporotic fracture: G1: 36 G2: 36 Season: NR	Serum 25(OH)D change at 1 year (nmol/L) G1: 18.0 ± 18.9 G2: 114.7 ± 34.6		One participant with serum calcium 2.77 mmol/L was erroneously excluded and did not complete the study (the respective group was not reported). A control value after one week was 2.39 mmol/L.	
Heaney et al. (2003) USA Latitude 41.2° N ≈20 wk Private funding	RCT (parallel) Inclusion criteria: Men in good general health, who habitually consumed no more than one serving of milk/d and who did not take a vitamin supplement. Exclusion criteria:	Sex: Men Age (y) All: 38.7 ± 11.2 Serum 25(OH)D (nmol/L) based on analysed doses G1: 70.2 ± 23.4 G2: 72.1 ± 16.0 G3: 69.3 ± 16.7 G4: 65.6 ± 24.2	Vitamin D_3 Doses (labeled) G1: placebo G2: vitamin D_3 1000 IU/d /25 μg/d G3: vitamin D_3 5000 IU/d /125 μg/d G4: vitamin D_3 10 000 IU/d / 250 μg/d	Elevated serum calcium above the upper limits of normal: - safety measure - total serum calcium measured at each visit - defined as >2.6 mmol/L	**Elevated serum calcium,** *n* of events Stated: Serum calcium in G1 and G2 did not change significantly from baseline (mean 9.6 mg/ dL [2.65 mmol/L]) at any time point at either dose. No value rose above the upper limit of normal.	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	Men who, over the 5-mo course of the study were planning a winter vacation to a location at which either the altitude or the latitude would be predicted to result in significant cutaneous vitamin D synthesis from solar radiation (e.g., a mountain ski resort or a Gulf Coast locale). N participants, randomised/completed/ analysed: All: 67/NR/NR	Assay: RIA BMI (kg/m^2) All: 26.2 ± 2.4 Ethnicity [assumed]: Caucasian Smoking status: NR Alcohol use: NR Health status: Healthy men Season: Winter months of 2 successive years (late Oct to late Feb or early Mar of each year)	Analysed vitamin D_3 doses G2: 836 IU / 20.9 µg/d G3: 5500 IU / 137.5 µg/d G4: 11 000 IU/d / 275 µg/d Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – vitamin D_3/placebo: NR Serum 25(OH)D, change at equilibrium (nmol/L) based on analysed doses: G1: −11.4 ± 17.7 G2: 12.0 ± 16.2 G3: 91.9 ± 37.6 G4: 159.4 ± 62.4			
Hin et al. (2016) **BEST-D** United Kingdom Latitude [assumed] 51° N to 58° N 1 yr Mixed funding	RCT (parallel) Inclusion/exclusion criteria: Individuals who were ambulatory, living in the community and not currently taking more than 400 IU (10 µg) vitamin D_3 daily were eligible to participate. N participants,	Sex (% Females) G1: 49.0 G2: 50.0 G3: 49.0 Age (y) G1: 72 ± 6 G2: 72 ± 6 G3: 71 ± 6 Serum 25(OH)D (nmol/L) G1: 47 ± 1.5 G2: 55 ± 2.2	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 2000 IU/d / 50 µg/d G3: vitamin D_3 4000 IU/d / 100 µg/d Background vitamin D intake: NR Background calcium intake (mg/d)	Elevated plasma levels of albumin-corrected calcium: - safety measure - percentage of participants with calcium above the reference interval at 1, 6 and 12 months - defined as >2.55 mmol/L	**Elevated plasma levels of albumin-corrected calcium above the normal range,** *n* of events Stated: At randomization, albumin-corrected calcium was mildly elevated (>2.55 mmol/L) in 8 participants (6 who were subsequently allocated vitamin D_3 and	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	randomised/completed/ analysed: G1: 101/95/101 G2: 102/98/102 G3: 102/97/102	G3: 49 ± 1.5 Assay: CLIA (Beckman Coulter Ltd., High Wycombe, England) BMI (kg/m^2) G1: 28 ± 5 G2: 27 ± 4 G3: 27 ± 5 Ethnicity [assumed]: Caucasian Smoking status (%) Current smokers: G1: 7 G2: 7 G3: 7 Alcohol use: NR Health status: Included a substantial number of healthy older people who are the group most at risk of osteoporotic fractures Season: NR	G1: 713 ± 302 G2: 695 ± 292 G3: 724 ± 287 Compliance Pill count method – vitamin D_3/placebo (%) At 6 mo G1: 87 G2: 93 G3: 93 At 12 mo G1: 85 G2: 92 G3: 90 Serum 25(OH)D at 12 mo (nmol/L) G1: 53 ± 16 G2: 102 ± 25 G3: 137 ± 39		two who were subsequently allocated placebo). By 12 mo, there were no new cases with elevated plasma levels of albumin-corrected calcium. Among the 8 participants who had marginally elevated plasma levels of albumin-corrected calcium at baseline, 6 (5 allocated vitamin D_3 and 1 allocated placebo) still had elevated levels at 12 mo, but none was considered clinically significant.	
Johnson et al. (2022) **D2d study** USA Latitude [assumed] 42.3° N 3 yr	RCT(parallel) Inclusion criteria: Eligible participants met at least two of three glycemic criteria for prediabetes as defined by the 2010 American Diabetes Association (ADA) guidelines. Other inclusion criteria were age greater	Sex (% Females) G1: 45.0 G2: 44.7 Age (y) G1: 60.4 ± 10.0 G2: 59.6 ± 9.9 Serum 25(OH)D (ng/mL) G1: 28.2 ± 10.1 ng/mL [70.5 ± 25.3 nmol/L]	Vitamin D_3 Doses (labeled) G1: placebo G2: vitamin D_3 4000 IU [100 μg/d] Analysed doses: Doses were analysed but the results were not provided Stated: To optimize safety, participants were	Hypercalcaemia: - secondary outcome - serum calcium was measured at month 3, and annually thereafter - defined as serum calcium value (uncorrected for albumin concentration) was > the site's clinical laboratory upper level of normal	**Hypercalcaemia**, *n* of participants There were 16 cases in G1 and 20 cases in G2 with new-onset hypercalcaemia on initial testing; on repeat testing, only 10 cases were confirmed, 4 in G1 and 6 in G2.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Mixed funding	than or equal to 30 years (25 years for American Indians, Alaska Natives, Native Hawaiians, or other Pacific Islanders) and body mass index (BMI) of 24–42 kg/m^2 (22.5–42 kg/m2 for Asian Americans) Exclusion criteria: A low serum 25 hydroxyvitamin D (25[OH] D) concentration was not an inclusion criterion. Key exclusion criteria included use of diabetes or weight-loss medications or a history of hyperparathyroidism, nephrolithiasis, hypercalcaemia, chronic kidney disease (defined as estimated glomerular filtration rate [eGFR] <50 mL/min/1.73 m2), calcium-to-creatinine ratio greater than 0.275 at baseline, or bariatric surgery. Persons were also excluded for use of supplements containing total doses of vitamin D higher than 1000 IU/day or total calcium higher than 600 mg/day.	G2: 27.7 ± 10.2 ng/mL [69.3 ± 25.5 nmol/L] Assay: LC–MS/MS with calibrators that are traceable to the National Bureau of Standards and Technology and validated by quarterly proficiency testing program administered by the Vitamin D External Quality Assessment scheme (DEQAS, United Kingdom) BMI (kg/m^2) G1: 32.1 ± 4.4 G2: 32.0 ± 4.5 Ethnicity (%) Asian: G1: 5.3 G2: 5.5 Black or African American G1: 26.0 G2: 24.9 White: G1: 66.5 G2: 66.9 Other G1: 2.3 G2: 2.8 Hispanic or Latino G1: 8.7 G2: 9.9 Smoking status: NR Alcohol use: NR	asked to limit calcium supplements to 600 mg/d Background vitamin D intake IU/d G1: 316 ± 397 IU/d [7.9 ± 9.9 μg/d] G2: 310 ± 401 IU/d [7.8 ± 10.0 μg/d] Background calcium intake (mg/d) G1: 107 ± 176 G2: 100 ± 175 Compliance Pill count method – vitamin D_3/placebo (%) All: 84.1	and ≥ the upper level of normal plus 1 mg/dL If serum calcium (uncorrected for albumin concentration) was > the site's clinical laboratory upper level of normal and ≥ the upper level of normal plus 1 mg/dL, testing was repeated within 6 weeks. If the repeat serum calcium value was > the site's clinical laboratory upper level of normal, the participant was confirmed to have met the outcome of hypercalcaemia; study pills were stopped, and the participant was referred to their health care provider. If the first measurement of serum calcium was > the upper level of normal plus 1 mg/dL [0.25 mmol/L], no repeat testing was required and the participant was considered to have met the outcome of hypercalcaemia; study pills were stopped, and the participant was referred to their health care provider. Hypercalciuria: - secondary outcomes - urine Ca/Cr ratio was measured at month 3, and annually thereafter - defined as >0.375	**Hypercalcaemia**, IRR (95% CI) G2 vs G1: 1.49 (0.42, 5.27) **Hypercalciuria,** *n* of participants There were 10 cases in G1 and 11 cases in G2 with new-onset hypercalciuria on initial testing; on repeat testing, only 2 cases were confirmed, 1 in each group **Hypercalciuria**, IRR (95% CI) G2 vs G1: 0.99 (0.06, 15.86)	

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	N participants, randomised/completed/analysed: G1: 1212/1119/1212 G2: 1211/1123/1211	Health status: Overweight/obese persons with prediabetes Season: NR		If urine Ca/Cr ratio was >0.375, testing was repeated within 4 weeks. If repeat urine Ca/Cr ratio remained >0.375, then the participant was considered to have met the outcome of hypercalciuria; study pills were stopped, and the participant was referred to their health care provider.		
Jorde et al. (2008); Sneve et al. (2008) Norway Latitude [assumed] 69.6° N 1 yr Mixed funding	(RCT parallel) Inclusion criteria: Males and females, 21–70 years old, with BMI between 28.0 and 47.0 kg/m2 were included. If the serum calcium was in the range 2.50–2.55 mmol/l, serum PTH had to be below 5.0 pmol/l. Exclusion criteria: Subjects with diabetes or a history of coronary infarction, angina pectoris, stroke, renal stone disease, or sarcoidosis were excluded. Subjects with a weight loss of more than 10% of total body weight during the last 6 months, those using anti-depressant drugs or weight reducing drugs, those participating in an	Sex (% Females) G1: 65.8 G2: 64.3 G3: 62.7 Age (y) G1: 48.9 ± 11.0 G2: 47.6 ± 11.9 G3: 46.4 ± 11.3 Serum 25(OH)D (nmol/L) G1: 53.2 ± 15.4 G2: 51.4 ± 18.4 G3: 54.5 ± 16.7 Assay: ECLIA, using an automated clinical chemistry analyser (Modular E170; Roche Diagnostics) BMI (kg/m^2) G1: 35.1 ± 3.8 G2: 34.4 ± 3.9 G3: 35.0 ± 4.1 Ethnicity [assumed]: Caucasian	Vitamin D_3 Doses G1: Double placebo + calcium 500 mg/d G2: Vitamin D_3 20 000 IU/wk [500 μg/wk = 71.43 μg/d] + placebo + calcium 500 mg/d G3: Double vitamin D_3 20 000 IU/wk [1000 μg/wk = 142.86 μg/d] + calcium 500 mg/d Background vitamin D intake μg/d G1: 8.9 ± 6.2 G2: 9.1 ± 7.0 G3: 9.0 ± 6.7 Background dietary calcium intake (mg/d) G1: 955 ± 390 G2: 943 ± 438 G3: 922 ± 368	Hypercalcaemia: - safety measure - serum calcium drawn after 3, 6, and 9 mo - defined as >2.59 mmol/L	One participant in G2 had an increased serum calcium level to 2.62 mmol/L after 6 mo, and the retest value was 2.60 mmol/L. The participant was excluded from the study. Serum calcium then normalised. 1 participant in G1 and 3 participants in G3 had transient increases in serum calcium at 6 mo (for 2 of them, as follows and they completed the study: "During the study, only seven subjects reached a serum calcium value above 2.59 mmol/l, which was our predefined hypercalcaemia threshold. Two subjects, one given placebo, developed PHPT	**2**

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	organized weight loss program, pregnant or lactating women, women planning to become pregnant in the next 12 months, women below the age of 50 years without adequate contraception contraceptive pills, implantable subdermal contraceptive rods, contraceptive dermal patches, injectable contraceptives, vaginal contraceptive rings, or intrauterine devices, with pearl index <1.0), subjects with serum calcium >2.55 mmol/l, males with serum creatinine >129 mmol/l, and females with serum creatinine >104 mmol/l were not included. N participants, randomised/completed/ analysed: G1: 149/112/112 G2: 143/106/106 G3: 153/116/116	Smoking status (%) Current smokers: G1: 21.5 G2: 21.7 G3: 22.9 Alcohol use: NR Health status: Healthy overweight and obese men and women Season: Summer and winter	Compliance Pill count method – vitamin D_3/placebo (%) All: 95 Pill count method - calcium (%) G1: 83 G2: 85 G3: 81 Serum 25(OH)D at 12 mo (nmol/L) G1: 50.0 (20.3–99.8) G2: 87.8 (51.5–162.3) G3: 112.1 (46.7–193.4)		and, in retrospect, their baseline serum calcium and PTH levels, although not outside the present limits for inclusion, indicated a disturbed calcium metabolism."). Serum calcium levels (mmol/L) in each hypercalcaemic cases by their respective groups G1: at screening 2.46; at 6 mo 2.60; retest 2.50 G2: at screening 2.30; at 6 mo 2.62; retest 2.60 G3: at screening 2.24; at 9 mo 2.61; retest 2.38 G3: at screening 2.31; at 3 mo 2.61; retest 2.39 G3: at screening 2.29; at 3 mo 2.61; retest 2.53	
Mastaglia et al. (2006) Argentina	RCT (parallel) Inclusion criteria:	Sex: Females Age (y) G1: 61.9 (54.5–67.0)	Vitamin D_2 Doses (labeled) G1: calcium 500 mg/d	Hypercalcaemia: - safety measure - serum calcium measured at baseline and at 1, 2 and 3 mo	**Hypercalcaemia** No individual value in any of the three groups was above the upper limit of	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Latitude 34° S 3 mo Private funding	50–70 years old; menopause had started at least 1 year prior to the study; lived in Buenos Aires Exclusion criteria: treatment with vitamin D or any other medication known to affect mineral metabolism, within 12 months prior to the study; a health condition which rendered administration of vitamin D unadvisable (renal lithiasis, tumors) or affecting vitamin D metabolism (hepatic disease, renal insufficiency). N participants, randomised/completed/ analysed: All: 45 G1: NR/13/13 G2: NR/13/13 G3: NR/12/12	G2: 65.0 (57.0–67.0) G3: 60.0 (56.2–66.2) Serum 25(OH)D (nmol/L) G1: 45.0 (31.2–61.2) G2: 42.0 (23.7–45.0) G3: 32.5 (27.5–37.5) Assay: RIA (Diasorin) BMI (kg/m^2): G1: 25.8 (23.2–28.6) G2: 27.4 (25.0–31.7) G3: 25.9 (22.4–30.4) Ethnicity [assumed]: Caucasian Smoking status: NR Alcohol use: NR Health status: Osteopenic/ osteoporotic women Season: winter and spring	G2: vitamin D_2 125 μg/ d + calcium 500 mg/d G3: vitamin D_2 250 μg/ d + calcium 500 mg/d Analysed vitamin D_2 doses: 62.5 μg per drop (patients in G2 received two daily drops (131 μg/ d) and those in G3 received four daily drops (262 μg/d)). Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method (%) G1: NR G2: 89 ± 11 G3: 92 ± 10 Serum 25(OH)D at 3 mo (nmol/L): G1: 55.0 (72.5–68) G2: 77.5 (66.2–156.2) G3: 97.7 (79.3–123.1)	- defined as >10.5 mg/dl [>2.625 mmol/L] Hypercalciuria: - safety measure - 24-h urine collected at baseline and at 1, 2 and 3 mo - defined as 24-h urine calcium >250 mg or Ca/Cr ratio >0.37 mg/mg.	the normal range (10.5 mg/dl [2.625 mmol/L]). **Hypercalciuria** One patient in G2 exhibited values >250 mg/24 h at 2 and 3 mo. One patient in G3 showed an increase from a baseline value of 229–278 mg/24-h after 3 mo treatment. One patient in G1 exhibited hypercalciuria (>250 mg/24 h) at 3 mo. Urinary calcium excretion was below 250 mg/24-h in all the remaining cases. There were no differences among groups at any of the studied times points when comparing the number of patients with urinary calcium levels >250 mg/24-h. None of the subjects showed a calciuria/creatininuria ratio >0.37 mg/mg during the follow-up.	
Ponda et al. (2012) USA	RCT (parallel) Inclusion criteria:	Sex (% Females) G1: 45 G2: 45	Vitamin D_3 Doses G1: placebo	Serum calcium: - secondary outcome	Serum calcium levels remained below the upper limit of normal for	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Latitude [assumed] 40.7° N 8 wk Public funding	Age 18–85 y; 25(OH)D level ≤ 20 ng/mL [50 nmol/L] and at least 1 of the following cardiovascular risk factors: BMI >30 kg/m^2, HDL <40 mg/dL for men or < 50 mg/dL for women, hsCRP >2 mg/L, glomerular filtration rate 30 to 59 mL/min per 1.73 m2, a history of coronary artery disease, diabetes mellitus, or a 10-year Framingham risk score > 10%. Exclusion criteria: Taking >400 IU of vitamin D_2 or D_3, or any dose of activated vitamin D (1,25 (OH)D or its analogues) within 1 mo; triglycerides >400 mg/dL; serum calcium >10.5 mg/dL; serum phosphorus >5.5 mg/dL, a change in any lipid therapy within 1 month; glomerular filtration rate < 30 mL/min per 1.73 m^2. N participants, randomised/completed/ analysed: All: NR/151/151	Age (y) G1: 47.4 ± 12.8 G2: 48.4 ± 11.3 Serum 25(OH)D (ng/ml): G1: 14.1 ± 5.7 [35.25 ± 14.25 nmol/L] G2: 13.4 ± 5.3 [33.5 ± 13.25 nmol/L] Assay: CLIA (LIASON, Diasorin) BMI (kg/m^2): NR Ethnicity (%) Blacks: G1: 47 G2: 45 Other ethnicities: NR Smoking status: NR Alcohol use: NR Health status: Vitamin D-deficient; elevated risk for cardiovascular disease Season: NR	G2: vitamin D_3 5 x 10 000 IU/wk [5 x 250 μg/wk = 178.6 μg/d] Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method: NR Serum 25(OH)D at 8 wk (ng/mL) G1: 14.6 ± 6.2 [36.5 ± 15.5 nmol/L] G2: 43.0 ± 12.3 [107.5 ± 30.75 nmol/L]	- measured at baseline and at 4 and 8 weeks - cut-off for elevated levels defined as >10.5 mg/dl [>2.625 mmol/L].	all subjects throughout the study.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	G1: NR/NR/75 G2: NR/NR/76					
Rafii et al. (2019) USA Latitude [assumed] 40.6° N 1 yr Private funding	RCT (parallel) Inclusion criteria: 25–85 years; documented 25(OH)D < 30 ng/ml [75 nmol/L] within the preceeding 2 mo; not on vitamin D supplementation (other than what is contained in a daily multivitamin, which is around 600 IU). Exclusion criteria: History of malabsorption, chronic kidney disease stages 3 to 5, end-stage renal disease, hypercalcaemia, nephrolithiasis, alcohol/ drug abuse, steroid use, androgen deprivation therapy; active hyper- or hypothyroidism; active malignancy (other than nonmelanoma skin cancer; inability to comply with study requirements; use of immunomodulators, anticonvulsants, teroparatide, orlistat, or reinoids; pregnancy; contemplating pregnancy.	Sex (% Females) G1: 20 G2: 25 G3: 20 G4: 20 Age (y) G1: 53.48 ± 12.53 G2: 56.00 ± 12.47 G3: 56.64 ± 11.06 G4: 52.40 ± 13.00 Serum 25(OH)D (ng/mL) G1: 17.5 ± 5.5 [43.75 ± 13.75 nmol/L] G2: 18.7 ± 6.2 [46.75 ± 15.5 nmol/L] G3: 14.4 ± 5.9 [36 ± 14.75 nmol/L] G4: 14.8 ± 5.7 [37 ± 14.25 nmol/L] Assay: CLIA (LIAISON, Diasorin) BMI (kg/m^2) G1: 32.33 ± 6.78 G2: 29.86 ± 6.13 G3: 32.06 ± 4.81 G4: 32.02 ± 4.86 Ethnicity (%) White: G1: 32 G2: 38 G3: 20	Vitamin D_2 and D_3 Doses G1: vitamin D_3 2000 IU/d [50 μg/d] + calcium 500 mg/d G2: vitamin D_3 3000 IU/d [75 μg/d] + calcium 500 mg/d G3: vitamin D_2 50 000 IU/wk [1250 μg/wk = 178.6 μg/d] + calcium 500 mg/d G4: vitamin D_2 50 000 IU two times/wk [2500 μg/wk = 57.1 μg/d] + calcium 500 mg/d Background vitamin D intake: NR Background calcium intake (mg/d) All: 300 on average. Compliance Pill count method (%) G1: 78.6 G2: 72.8 G3: 89.5 G4: 87.7 Serum 25(OH)D, mean change over 12 mo (ng/mL):	Hypercalcaemia: - secondary outcome - serum calcium was measured at baseline and at 1, 3, 5, 7, 9 and 12 mo - clinically significant hypercalcaemia defined as serum calcium level >1 ng/mL[1 mg/dl; 0.25 mmol/L] above the upper limit of normal; as reference range of 8.4–10.2 mg/dL [2.1–2.55 mmol/L] was provided, the cut-point was 2.7 mmol/L Elevated Ca/Cr ratio: - secondary outcome - urine Ca/Cr ratio from spot urine measured at baseline and at 1, 3, 5, 7, 9, and 12 mo - defined as Ca/Cr ratio >0.14 and >0.20.	**Hypercalcaemia** None of the subjects developed clinically significant hypercalcaemia. The highest calcium attained was 10.8 ng/mL [2.7 mmol/L], which occurred at visit 2 in an individual in G2. **Elevated urine Ca/Cr ratio** The percentage of subjects with Ca/Cr ratio >0.14 fluctuated throughout the study without a clear trend. G1 had the highest Ca/Cr ratio in all but the second visit. 23% of participants had a Ca/Cr ratio of >0.14 at baseline: this rose to 31% by 12 mo. The percentage of participants with Ca/Cr ratio >0.2, a value seen in those with hypercalciuria, rose from 9.4% at baseline to 12.9% at 12 mo. The highest Ca/Cr ratios were inexplicably seen with G1.	**3**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	N participants, randomised/completed/ analysed: G1: 25/16/24 G2: 25/17/24 G3: 25/16/25 G4: 25/16/25	G4: 24 Black: G1: 40 G2: 54 G3: 64 G4: 56 Hispanic: G1: 16 G2: 8 G3: 16 G4: 16 Asian: G1: 8 G2: 0 G3: 0 G4: 4 Other: G1: 4 G2: 0 G3: 0 G4: 0 Smoking status: NR Alcohol use: NR Health status: Vitamin D insufficient Season: NR	G1: 13.04 [32.6 nmol/L] G2: 33.58 [83.95 nmol/L] G3: 38.46 [96.15 nmol/L] G4: 56.76 [141.88 nmol/L]			
Rorie et al. (2014) USA Latitude [assumed] 41.2° N 12 wk	RCT (parallel) Inclusion criteria: A history of urticarial and/ or angioedema daily or almost daily for longer than 6 week; chronic urticaria having signs of dermatographism and/or	Sex (% Females) G1: 71.4 G2: 85.7 Age (y); mean, range G1: 43.1, 19–79 G2: 43.9, 20–72	Vitamin D_3 Doses G1: vitamin D_3 600 IU/d [15 μg/d] G2: vitamin D_3 4000 IU/d [100 μg/d]	Hypercalcaemia: - safety measure - blood was collected at enrollment and at 6 and 12 wk and processed for calcium - defined as serum calcium >10.3 mg/dL [2.575 mmol/L] Hypercalciuria:	**Hypercalcaemia** There was no evidence of hypercalcaemia. **Hypercalciuria** The 3 participants (2 in G1 and 1 in G2) had 1-time spot urine calcium level > 30 mg/dL	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
Private funding	delayed-pressure urticaria history of intolerance to nonsteroidal anti-inflammatory drugs were included but warned not to take this drug class (acetaminophen was allowed). Patients with a history of alcohol-exacerbating hives were included but were counselled to avoid alcohol. Exclusion criteria: Diagnosed with a pure physical urticaria, hereditary or acquired angioedema, hypercalcaemia (>10.3 mg/dL [2.5757.49 mmol/L]), renal insufficiency (glomerular filtration rate < 50 mL/min/1.73 m2), primary hyperparathyroidism, sarcoidosis, granulomatous disease, or malignancy or were pregnant or lactating. N participants, randomised/completed/analysed: G1: 21/17/17 G2: 21/21/21	Serum 25(OH)D (ng/mL), mean ± SE G1: 37.1 ± 3.4 ng/mL [92.8 ± 8.5 nmol/L] G2: 28.8 ± 2.2) ng/mL [72.0 ± 2.5 nmol/L] Assay: MS/MS BMI (kg/m^2) G1: 30.5 ± 6.32 G2: 30.6 ± 9.42 Ethnicity (%) White: G1: 90.4 G2: 95.2 African American: G1: 4.7 G2: 4.7 Asian: G1: 4.7 G2: 0 Smoking status (%) Current smokers: G1: 23.8 G2: 28.5 Former smokers: G1: 52.3 G2: 47.6 Alcohol users (%) G1: 42.8 G2: 14.2 Health status: Adult patients with physician-diagnosed	Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – vitamin D_3 (%) All: >80 Stated: Excellent compliance, with only 1 subject in G1 showing <80% compliance. Serum 25(OH)D at 12 wk (ng/mL), mean ± SE G1: 35.8 ± 2.3 ng/mL [89.5 ± 5.8 nmol/L] G2: 56.0 ± 3.9) ng/mL [140.0 ± 9.8 nmol/L]	- safety measure - spot urine for urine calcium was collected for safety end points -unclear at which time points. - defined as spot urine calcium >30 mg/dL [7.49 mmol/L]. Specific stopping rules and discontinuation of the study included a serum calcium level > 10.3 mg/dL [2.575 mmol/L]. Safety guidelines were implemented if the spot urine calcium level was > 30 mg/dL.	[7.49 mmol/L], which resolved on repeat measurement.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
		Chronic urticaria with or without angioedema Season: NR				
Schwartz et al. (2016) USA Latitude [assumed] 37.7° N 16 wk Mixed funding	(RCT parallel) Inclusion/exclusion criteria: No hypercalcaemia, history of hypercalcaemia, uncontrolled thyroid or parathyroid disorders, severe renal failure (estimated glomerular filtration rate (eGFR) <30 mL/min per 1.73 m2), active malignancies (except nonmelanoma skin cancer), intestinal bypass surgery or small bowel resection, granulomatous diseases, contraindications or allergy to vitamin D, osteoporosis, or a history of fractures; not receiving more than 800 IU/d [20 μg/d] of vitamin D, or treatment for severe vitamin D deficiency or an investigational agent in the prior 6 months. They received no vitamin D supplements (vitamin D naive) or had stable vitamin D doses for longer	Sex (% Females) G1: 70.0 G2: 63.2 G3: 65.0 G4: 76.9 Age (y) G1: 84.9 ± 8.7 G2: 85.9 ± 8.5 G3: 89.5 ± 6.6 G4: 90.1 ± 6.6 Serum 25(OH)D (ng/mL) G1: 29.4 ± 10.4 ng/mL [73.5 ± 26.0 nmol/L] G2: 28.9 ± 10.4 ng/mL [72.3 ± 26.0 nmol/L] G3: 29.0 ± 9.9 ng/mL [72.5 ± 24.8 nmol/L] G4: 32.3 ± 6.0 ng/mL [80.8 ± 15.0 nmol/L] Assay: LC–MS/MS (Mayo Clinical Laboratories, Rochester, MN). BMI (kg/m^2) G1: 27.5 ± 6.8 G2: 27.6 ± 5.3 G3: 27.0 ± 5.4 G4: 28.2 ± 5.7	Vitamin D_3 Doses (labeled) G1: vitamin D_3 800 IU/d [20 μg/d] G2: vitamin D_3 2000 IU/d [50 μg/d] G3: vitamin D_3 4000 IU/d [100 μg/d] G4: vitamin D_3 50 000 IU/wk [1250 μg/wk = 178.6 μg/d] Analysed doses G1: 858 ± 29 IU [21.5 ± 0.73 μg/d] and 861 ± 45 IU [21.5 ± 1.1 μg/d] G2: 2467 ± 69 [61.7 ± 1.7 μg/d] and 2482 ± 73 IU [62.0 ± 1.8 μg/d] G3: 4839 ± 202 [121.0 ± 5.1 μg/d] and 4807 ± 108 IU [120.2 ± 2.7 μg/d] G4: 68 354 ± 2296 IU [1 708 ± 57.4 μg/d] and 57 542 ± 356 IU [1 439 ± 8.9 μg/d];	Hypercalcaemia: - safety measure - unclear measurement frequency of albumin-adjusted serum calcium - no cut-off provided. However, chemistry panels were analysed at baseline, midstudy, and study end, probably including serum calcium	**Hypercalcaemia** In the interim safety analysis, hypercalcaemia did not occur, nor were corrected calcium concentrations changed by more than 5% (maximum changes were 0.5 and 0.6 mg/dL [0–125 - 0.15 mmol/L] in two participants). Adverse effects over intervention period: Hypercalcaemia occurred in one participant assigned to 4 000 IU/d [100 μg/d] that resolved with discontinuation of supplemental calcium.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	than 2 months before entry. N participants, randomised/completed/ analysed: G1: 23/20/20 G2: 20/19/19 G3: 24/20/20 G4: 14/13/13	Ethnicity (%) White: G1: 95 G2: 100 G3: 100 G4: 100 Asian: G1: 5 G2: 0 G3: 0 G4: 0 Smoking status: NR Alcohol use: NR Health status: Clinically stable long-term stay nursing home residents Season: NR	at study initiation and end, respectively. Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – vitamin D_3 (%) All: 96 ± 7 Serum 25(OH)D at 16 wk (ng/mL) G1: 33 ± 6 ng/mL [82.5 ± 15.0 nmol/L] G2: 34 ± 6 ng/mL [85.0 ± 15.0 nmol/L] G3: 43 ± 10 ng/mL [107.5 ± 25.0 nmol/L] G4: 61 ± 14 ng/mL [152.5 ± 35.0 nmol/L]			
Shirvani et al. (2020) USA Latitude [assumed] 42.3° N 6 mo Unclear funding	(RCT parallel) Inclusion criteria: Healthy young black and white adults with a BMI < 30 kg/m^2 without disorders or medications affecting vitamin D metabolism. Exclusion criteria:	Sex (% Females) G1: 66.7 G2: 61.5 G3: 62.5 Age (y) G1: 26.3 ± 2.0 G2: 25.3 ± 2.1 G3: 26.1 ± 2.0 Serum 25(OH)D (ng/mL)	Vitamin D_3 Doses G1: vitamin D_3 600 IU/d [15 μg/d] G2: vitamin D_3 4000 IU/d [100 μg/d] G3: vitamin D_3 10 000 IU/d [250 μg/d] Background vitamin D intake: NR	Elevated serum calcium - safety measure - serum calcium measured at baseline and every 8 wk - defined as serum calcium >10.5 mg/dL [2.625 mmol/L] - the participation of an individual who discovered to have elevated serum calcium	Serum calcium levels remained normal for all study participants and no untoward toxicity was observed.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	History of elevated serum calcium (>10.5 mg/dL [2.625 mmol/L]); vitamin D supplementation with a dose of 600 IU/day or more; direct exposure to UV during the past month for greater than eight hours; any kind of malabsorption; history of chronic or acute renal or hepatic disease; current antiseizure medications or glucocorticoids; pregnant/ lactating women; and reluctance to consent to the study. N participants, randomised/completed/ analysed: All: 33 G1: NR/9/9 G2: NR/13/13 G3: NR/8/8	G1: 17.1 ± 5.9 ng/mL [42.8 ± 14.8 nmol/L] G2: 22.5 ± 5.7 [56.3 ± 14.3 nmol/L] G3: 17.8 ± 3.3 [44.5 ± 8.3 nmol/L] Assay: LC–MS/MS BMI: NR Ethnicity (%) Non-White: G1: 66.7 G2: 38.5 G3: 50.0 Smoking status: NR Alcohol use: NR Health status: Healthy adults with insufficient serum 25 (OH)D Season: Recruitment occurred from Oct to March	Background calcium intake: NR Compliance Pill count method – vitamin D_3: NR Serum 25(OH)D, average increase at 24 wk (ng/ mL) G1: 7 ng/ml (18 nmol/l) G2: 18 ng/ml (45 nmol/l) G3: 61 ng/ml (153 nmol/l)	was immediately discontinued and the primary care physician was informed.		
Vieth et al. (2001) Canada Latitude: 43° N 2–5 mo Private funding	(RCT parallel) Inclusion/exclusion criteria: Generally healthy volunteers, most of whom worked in the clinical laboratory departments of 2 Toronto hospitals.	Sex: % (Females) G1: 69.7 G2: 64.3 Age (y) G1: 41.6 (18–53) G2: 39.9 (23–56) Serum 25(OH)D (nmol/L): G1: 43.3 ± 16.8 G2: 37.9 ± 13.4	Vitamin D_3 Doses G1: vitamin D_3 25 µg/d / 1000 IU/d G2: vitamin D_3 100 µg/d / 4000 IU/d Background vitamin D intake: NR	Hypercalcaemia: - primary outcome - serum calcium measured at baseline and at 0.5, 1, 2, 3, 4, and 5 mo - defined as serum calcium concentration >2.75 mmol/L Hypercalciuria: - primary outcomes	**Hypercalcaemia** Serum calcium concentrations remained within the reference range (2.2–2.6 mmol/L) during the study in all subjects in G1 and G2. **Hypercalciuria**	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
	N participants, randomised/completed/ analysed: All:73 G1: NR/33 ≥ 1 mo; 26 at ≥3 mo; 15 at 5 mo/NR G2: NR/28 ≥ 1 mo; 25 ≥ 3 mo; 15 at 5 mo/NR [those who completed ≥1 mo were considered as the baseline population by the authors]	Assay: RIA (DiaSorin, Stillwater, MN) BMI: NR Ethnicity (%) White: G1: 66.6 G2: 71.4 Black: G1: 6.1 G2:10.7 Asian: G1: 27.3 G2: 17.9 Smoking status: NR Alcohol use: NR Health status: Healthy men and women. Season: Winter and summer; the study began between Jan and Feb.	Background calcium intake: NR Compliance Vials that contained the vitamin D_3 solutions were collected and subjects were given fresh vials: NR Peaked serum 25(OH)D at 3 mo (nmol/L) G1: 68.7 ± 16.9 G2: 96.4 ± 14.6 Stated that the concentrations remained relatively stable for the remainder of the study.	- second void urine sample for calcium was collected at baseline and at 0.5, 1, 2, 3, 4, and 5 mo - defined as a mean urinary Ca/Cr ratio >1.0 mmol or >0.37 mg	There were more urinary Ca/Cr excretion ratios >1.0 mmol/L in G2 (in one subject, 2 of 6 values were >1.0 during treatment) than in G1. The relative number of occurrences of hypercalciuria across the entire follow-up period was not significantly different between the 2 dosage groups.	
Wagner et al. (2016) Sweden Latitude [assumed] 59.3° N 8 wk Mixed funding	RCT (parallel) Inclusion criteria: 1) IFG, IGT, IFG + IGT, or drug-naïve diabetes at the screening OGTT (IFG = fasting plasma glucose [p-glucose] 6.1–6.9 mmol/L; IGT = 2-h p-glucose 7.8–11.0 mmol/L; diabetes = fasting p-glucose ≥7.0 mmol/L and/or 2-h p-glucose	Sex: % (Females) G1: 50.0 G2: 42.9 Age (y) G1: 67.0 (64.7–68.5) G2: 67.6 (63.4–68.8) Serum 25(OH)D (nmol/L) G1: 47 (42–53) G2: 42 (35–55) Serum 25(OH)D (nmol/L), season-adjusted	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 30 000 IU μg/wk [750 μg/wk = 107.1 μg/d] Background vitamin D intake: NR Background calcium intake: NR	Hypercalcaemia: - secondary outcome - serum calcium measured at baseline and at 4 and 8 wk - defined as ionized serum calcium >1.35 mmol/L.	No events of hypercalcaemia occurred in the study.	2

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	≥11.1 mmol/L) (22); 2) age ≥ 45 and ≤ 75 years, female or male; 3) BMI ≤32 kg/m2; 4) glycosylated haemoglobin (HbA1c) ≤7.9% (63 mmol/mol); 5) fasting p-glucose <9 mmol/L; 6) serum 25 (OH)D < 75 nmol/L (below normal lab reference); and 7) able and willing to perform tests and examinations specified in the protocol. Exclusion criteria: 1) antidiabetic medication of any kind; 2) anticipated change of concomitant medication that may interfere with glucose metabolism, such as systemic corticosteroids, nonselective b-blockers, monoamine oxidase inhibitors, and anabolic steroids; 3) treatment with any vitamin D preparation; 4) regular sunbathing in solarium; 5) hypercalcaemia at screening, defined as ionized s-calcium	G1: 43 (37–54) G2: 43 (36–50) Assay: CLIA (LIAISON, intra- and interassay coefficient of variation of 5 and 8–11%, respectively) BMI (kg/m^2) G1: 28.6 (26.4–29.9) G2: 28.3 (24.5–29.4) Ethnicity: White Smoking status NR Alcohol use: NR Health status: Participants with prediabetes or diet-treated type 2 diabetes. Season: Feb 2012 to May 2013	Compliance Pill count method – vitamin D_3/placebo (%): NR although stated in the method section that it was assessed. Serum 25(OH)D, change at 8 wk (nmol/L) G1: −1 (−3–5) G2: 41 (27–50) Adjusted serum 25(OH)D, change at 8 wk (nmol/L) G1: 0 (−7–11) G2: 42 (32–50)			

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
	>1.35 mmol/L; 6) hyperphosphatemia at screening, defined as s-phosphate >1.5 mmol/L; 7) sarcoidosis or other granulomatous disease; 8) treatment with phenytoin, barbiturates, rifampicin, isoniazid, cardiac glycosides, orlistat, or colestyramin (known to interfere with vitamin D metabolism); 9) impaired hepatic function, defined as alanine aminotransferase (ALT) three or more times the upper reference limit; 10) impaired renal function, defined as s-creatinine >133 mmol/L for males and > 115 mmol/L for females; 11) cardiac disease, defined as a) unstable angina pectoris, b) myocardial infarction within the last 6 months, or c) congestive heart failure New York Heart Association class III and IV; 12) cerebral stroke within the last 6 months; 13) uncontrolled treated/ untreated hypertension (systolic blood pressure ≥ 180 mmHg and/or diastolic					

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed	Results	RoB Tier
	blood pressure ≥ 110 mmHg); 14) cancer (except basal cell skin cancer or squamous cell skin cancer); 15) females of childbearing potential who were pregnant, breast-feeding, or intended to become pregnant or were not using adequate contraceptive methods; 16) known or suspected abuse of alcohol or narcotics; or 17) mental incapacity, unwillingness, or language barrier precluding adequate understanding or cooperation. N participants, randomised/completed/ analysed: G1: 22/21/21 G2: 22/22/22					
Wamberg et al. (2013) Denmark Latitude [assumed] 56.1° N 26 wk Unclear funding	(RCT parallel) Inclusion criteria: Women not planning pregnancy and reporting the use of safe contraception. Exclusion criteria: A history of diabetes, a fasting plasma glucose [7.0 mmol/L,	Sex: % (Females) G1: 73 G2: 69 Age (y) G1: 41.2 ± 6.8 G2: 39.5 ± 8.0 Serum 25(OH)D (nmol/L) G1: 34.6 ± 10.3 G2: 34.5 ± 10.8 Assay: LC–MS/MS	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 7000 IU (175 μg/d) Background vitamin D intake (μg/d) G1: 1.7 (1.4–3.1) G2: 2.1 (1.5–4.0)	Hypercalcaemia: - safety measure - serum calcium measured at baseline and at 26w; safety measures and adverse events measured at 2, 10, and 18 wk, apparently including serum ionized calcium - reference range for serum ionized calcium defined as 1.18–1.32 mmol/L	No subjects developed symptomatic hypercalcaemia during the study. Only one subject in G2 had plasma calcium just above the upper reference limit at one occasion.	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed	Results	RoB Tier
	hypercalcaemia, or impaired renal plasma creatinine [130 lmol/L) or hepatic function alanine aminotransferase [135 U/L); treatment with vitamin D within the last 3 mo; a history of sarcoidosis, osteomalacia, or alcohol or substance abuse. N participants, randomised/completed/ analysed: G1: 26/21/NR G2: 26/22/NR	BMI (kg/m^2) G1: 35.0 ± 3.2 G2: 36.1 ± 3.4 Ethnicity [assumed]: Caucasian Smoking status (%) Current smokers: G1: 19 G2: 27 Ex-smokers: G1: 27 G2: 31 Non-smokers G1:54 G2: 42 Alcohol use: Alcohol abusers were excluded. Health status: Healthy obese men and women Season: NR (Recruited from February 2010 until May 2011)	Background dietary calcium intake (mg/d) G1: 936 ± 389 G2: 992 ± 400 Compliance Pill count method – vitamin D_3/placebo (%) G1: 94 ± 8 G2: 95 ± 6 Serum 25(OH)D at 26 wk (nmol/L) G1: 46.8 ± 21.2 G2: 110 ± 17.3			

Abbreviations: BEST-D; the Biochemical Efficacy and Safety Trial of vitamin D; Ca/Cr: calcium to creatinine; CPBA: competitive protein binding assay; CI: confidence interval; CLIA: chemiluminescence immunoassay; CT: clinical trial; D2d: the Vitamin D and Type 2 Diabetes; G: group; ECLIA: electrochemiluminescence immune assay; HPLC–MS/MS: high performance liquid chromatography tandem mass spectrometry; IQR: inter quartile range; IRR: incidence rate ratio; LC–MS: liquid chromatography mass spectrometry; LC–MS/MS: liquid chromatography tandem mass spectrometry; MIDG, Maternal Vitamin D for Infant Growth; MS/MS: tandem mass spectrometry; RCT: randomised controlled trial; SE: Standard error; STURDY: Study To Understand Fall Reduction and Vitamin D in You; NR: not reported; OR: odds ratio; RIA: radioimmunoassay; ViDOS: Vitamin D Supplementation in Older Women; 25(OH)D: 25-hydroxyvitamin D.
a: the values have been reported as mean ± standard deviation or median (IQR) unless otherwise indicated; IQR = the 25th–75th percentiles or the length between the percentiles

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C.3. Intervention studies on the risk of bone fractures and falling

Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
Appel et al. (2021) **STURDY** USA Latitude 39° N 2 y (median 22.0 mo, range 0.4–31.3 mo) Mixed funding	RCT (Bayesian response-adaptive in dose-finding stage; parallel in best-dose stage) Inclusion criteria: Community-dwelling adults; aged ≥70 years; elevated fall risk (defined by self-report of 1 or more of the following: 2 or more falls or at least 1 injurious fall in the past year, fear of falling due to balance or walking problems, difficulty maintaining balance, and use of an assistive device when walking); low serum 25(OH)D levels (not defined). Exclusion criteria: Cognitive impairment; hypercalcaemia; kidney, bladder, or ureteral stones; use of supplemental vitamin D in doses higher than 1000 IU/d [25 μg/d] or calcium in doses higher than 1200 mg/d. Persons using 1000 IU/d [25 μg/d] or less of supplemental vitamin D had	Sex (% females) G1: 43.0 G2: 47.1 G3: 42.6 G4: 40.6 Age (y) G1: 77.5 ± 5.6 G2: 76.4 ± 4.8 G3: 77.3 ± 4.6 G4: 79.1 ± 5.9 Serum 25(OH)D (nmol/L) G1: 54.8 ± 31.2 G2: 56.3 ± 10.7 G3: 52.5 ± 13.1 G4: 53.6 ± 12.4 BMI (kg/m^2) G1: 30.3 ± 6.4 G2: 30.5 ± 5.3 G3: 30.7 ± 6.4 G4: 30.3 ± 6.2 Ethnicity (%) White: G1: 83.6 G2: 71.7 G3: 76.1 G4: 82.6 Black: G1: 15.6 G2: 25.0 G3: 22.4 G4: 14.5	Vitamin D_3 Doses Dose-finding stage: G1: vitamin D_3 200 IU/d [5 μg/d] G2: vitamin D_3 1000 IU/d [25 μg/d] G3: vitamin D_3 2000 IU/d [50 μg/d] G4: vitamin D_3 4000 IU/d [100 μg/d] Best-dose stage:[1,2)] G1: vitamin D_3 200 IU/d [5 μg/d] G2–G4: vitamin D_3 1000 IU/d [25 μg/d] [2)] Newly recruited participants and the participants in G3 and G4 were assigned vitamin D_3 1000 IU/d [25 μg/d]. Background vitamin D intake (IU/d) G1: 307 (647) IU/d [7.68 (16.2) μg/d] G2: 293 (702) IU/d [7.33 (17.6) μg/d] G3: 345 (779) IU/d [8.63 (19.5) μg/d] G4: 237 (520) IU/d [5.93 (13.0) μg/d]	**Falls** - defined as any fall, slip, or trip in which the participant lost his or her balance and landed on the floor or ground or at a lower level. - surveillance methods: monthly calendars, scheduled clinic visits and telephone calls, and ad hoc telephone contacts (participants were instructed to call the clinic if they fell); participants were asked to document on their study calendar each day whether a fall occurred and to mail their calendar each month - when a fall was reported, staff called the participant to obtain additional information. **Serious falls** - defined as a fall resulting in a dislocated, broken, or	Data extracted: ITT **Dose-finding stage - Falls** First fall during dose-finding, *n* of participants G1: 123 G2: 43 G3: 41 G4: 41 First fall during dose-finding, rate per 100 person-years (95% CI) G1: 88.3 (74.0, 105.4) G2: 70.5 (52.3, 95.0) G3: 103.0 (75.8, 139.9) G4: 99.4 (73.2, 135.0) First fall during dose-finding, HR (95% CI) G2 vs G1: 0.80 (0.57, 1.14) G3 vs G1: 1.20 (0.84, 1.71) G4 vs G1: 1.16 (0.82, 1.65) G3 vs G2: 1.50 (0.98, 2.31) G4 vs G2: 1.45 (0.94, 2.22) **Dose-finding stage – Serious falls** First serious fall during dose-finding, *n* of participants G1: 12 G2: 4 G3: 5 G4: 6	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	to agree to maintain this dosage. N participants, randomised/ completed/analysed Dose-finding stage (Oct 2015 to Mar 2018): G1: 256/NR/NR G2: 121/118/121 G3: 68/66/68 G4: 69/66/69 Best-dose stage[1)] (Apr 2018 to May 2019): G1: +38 = 339/329/339 G2–G4: +91 = 349/338/ITT 349, PP 308 [1)] New recruited participants + participants participating in the dose-finding stage; recruitment was ongoing throughout Oct 2015 to Feb 2019; not all analysed participants underwent at least 12 mo intervention, as interpreted from Supplementary Tables 2 and 3.	Other: G1: 2.0 G2: 7.5 G3: 3.0 G4: 2.9 Smoking status: NR Alcohol use: NR Health status – Frailty (%) Robust: G1: 31.2 G2: 25.8 G3: 36.8 G4: 26.1 Pre-frail: G1: 57.8 G2: 60.8 G3: 52.9 G4: 58.0 Frail: G1: 10.9 G2: 13.3 G3: 10.3 G4: 15.9 Fallen at least once in prior year (%) G1: 63.3 G2: 62.0 G3: 63.2 G4: 68.1 Season: Oct 2015 to May 2019.	Background vitamin D supplement use (%) G1: 37.5 G2: 39.7 G3: 39.7 G4: 33.3 Background calcium intake: NR <u>Compliance</u> Pill count method (%) At least 80% of daily pills taken: G1: 75.8 G2–G4: 72.5 50%–79% of daily pills taken: G1: 13.9 G2–G4: 11.5 25%–49% of daily pills taken: G1: 4.4 G2–G4: 8.0 Less than 25% of daily pills taken: G1: 5.9 G2–G4: 8.0 Serum 25(OH)D (nmol/L) During dose-finding at 12 mo: G1: 68.3 ± 17.9 G2: 81.0 ± 16.7 G3: 83.3 ± 17.9 G4: 123.2 ± 36.9	fractured body part, or a fall associated with a serious adverse event. **** Assay of serum 25 (OH)D: HPLC–MS/MS	First fall with hospitalization during dose-finding, *n* of participants G1: 6 G2: 2 G3: 5 G4: 1 First serious fall during dose-finding, rate per 100 person-years (95% CI) G1: 5.4 (3.1, 9.5) G2: 4.9 (1.8, 13.1) G3: 7.3 (3.0, 17.5) G4: 8.9 (4.0, 19.9) First fall with hospitalization during dose-finding, rate per 100 person-years (95% CI) G1: 2.7 (1.2, 5.9) G2: 2.4 (0.6, 9.6) G3: 7.3 (3.0, 17.5) G4: 1.4 (0.2, 10.1) First serious fall during dose-finding, HR (95% CI) G2 vs G1: 0.91 (0.29, 2.81) G3 vs G1: 1.37 (0.48, 3.89) G4 vs G1: 1.68 (0.63, 4.48) First fall with hospitalization during dose-finding, HR (95% CI) G2 vs G1: 0.86 (0.17, 4.29) G3 vs G1: 2.84 (0.87, 9.30) G4 vs G1: 0.55 (0.07, 4.60)	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
			During dose-finding + best dose stages at 24 mo: G1: 70.4 ± 16.6 G2–G4: 99.2 ± 29.9		**Dose-finding + best-dose stages - Falls** First fall over 2 y, *n* of participants G1: 208 G2–G4: 215 First fall over 2 y, rate per 100 person-years (95% CI) G1: 74.8 (65.3, 85.7) G2–G4: 77.4 (67.8, 88.5) First fall over 2 y, HR (95% CI) G2–G4 vs G1: 1.05 (0.87, 1.27) Data extracted: PP **Dose-finding + best-dose stages- Falls** First fall over 2 y, *n* of participants G1: 208 G2–G4: 157 First fall over 2 y, rate per 100 person-years (95% CI) G1: 74.8 (65.3, 85.7) G2–G4: 77.6 (66.3, 90.7) First fall over 2 y, HR (95% CI) G2–G4 vs G1: 0.96 (0.78, 1.18)	
Bischoff-Ferrari et al. (2020); Bischoff-Ferrari et al. (2022)	RCT (parallel) Inclusion criteria:	Sex (% females) G1–G4: 61.4 G5–G8: 62.0	Vitamin D_3 Doses	**Fractures** - nonvertebral - confirmed by x-ray reports or medical	Data extracted: unclear **Fractures**	**1 for fractures; 2 for falls**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
DO-HEALTH Switzerland, Germany, Austria, France, and Portugal Latitude NA 3 y Mixed funding	Generally healthy, ≥70 years old and community dwelling; no major health events (i.e. cancer or myocardial infarction) in the 5 y prior to enrolment; sufficient mobility to come to the study centres without help; a Mini-Mental State Examination score of ≥24; able to swallow study capsules; able and willing to participate, sign informed consent (including consent to analyze all samples until drop-out or withdrawal) and cooperate with study procedures. Recruitment was conducted with the goal of including ≥40% of participants with a history of falling in the prior 12 mo to increase representation of older adults at higher risk of frailty. Exclusion criteria: Taking >1000 IU/d [25 μg/d] of vitamin D in supplements during the 36 mo prior to enrolment or being unwilling to limit vitamin D supplement	Age (y) G1–G4: 74.9 ± 4.4 G5–G8: 75.0 ± 4.5 Serum 25(OH)D (ng/mL) G1–G4: 22.4 ± 8.5 ng/mL [56 ± 21.25 nmol/L] G5–G8: 22.4 ± 8.4 ng/mL [56 ± 21 nmol/L] BMI (kg/m^2) G1–G4: 26.2 ± 4.2 G5–G8: 26.5 ± 4.4 Ethnicity [assumed]: Majority Caucasian Smoking status: NR Alcohol use: NR Health status: Generally healthy Fallen in prior year (%) G1: 42.3 G2: 41.4 Season: NR	G1: double placebo + CEP G2: double placebo + SHEP G3: vitamin D placebo + omega-3 fatty acids 1 g/d + CEP G4: vitamin D placebo + omega-3 fatty acids 1 g/d + SHEP G5: vitamin D_3 2000 IU/d [50 μg/d] + omega-3 fatty acid placebo + CEP G6: vitamin D_3 2000 IU/d [50 μg/d] + omega-3 fatty acid placebo + SHEP G7: vitamin D_3 2000 IU/d [50 μg/d] + omega-3 fatty acids 1 g/d + CEP G8: vitamin D_3 2000 IU/d [50 μg/d] + omega-3 fatty acids 1 g/d + SHEP CEP = control exercise program; SHEP = simple home strength exercise program. Background vitamin D intake: NR Background vitamin D supplement use, ≥800 IU [20 μg] (%) G1–G4: 11.7	records describing an x-ray report or repair of the fracture. **Falls** - defined as unintentionally coming to rest on the ground, floor, or other lower level - recorded at each 3-mo in-person contact by asking participants whether they had sustained a fall in the last 3 mo; was supported by a fall diary - fall protocol collected information on the fall circumstances, related injuries, treatment, and related health-care utilization. **Injurious falls** - defined as falls leading to any injury. **** Assay of serum 25 (OH)D: LC–MS	Fractures over 3 years, *n* of events G1–G4: 127 G5–G8: 129 Fractures, unadjusted IRR (99% CI) G5–G8 vs G1–G4: 1.02 (0.74, 1.40) Fractures, adjusted IRR (99% CI) [3)] G5–G8 vs G1–G4: 1.03 (0.75, 1.43) Data extracted: ITT **Falls** Falls over 3 years, *n* of events G1–G4: 1673 G5–G8: 1660 ≥1 fall over 3 years, *n* of participants G1–G4: 654 G5–G8: 657 Falls, unadjusted IRR (95% CI) G5–G8 vs G1–G4: 1.00 (0.90, 1.12) Falls, adjusted IRR (95% CI) [4)] G5–G8 vs G1–G4: 1.03 (0.92, 1.14)	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	intake to 800 IU/d [20 μg/d] and calcium supplementation to 500 mg/d during trial; taking omega-3 supplements during the 3 mo prior to enrolment and/or being unwilling to avoid them during the trial; use of any active vitamin D metabolite, PTH treatment, or calcitonin at baseline and unwillingness to forego these treatments during the course of the trial; current or recent (previous 4 mo) participation in another clinical trial, or plans of such participation in the next 3 years (corresponding to DO-HEALTH length); presence of the following diagnosed health conditions in the last 5 years: history of cancer (except non-melanoma skin cancer), myocardial infarction, stroke, transient ischemic attack, angina pectoris, or coronary artery intervention; severe renal impairment (creatinine clearance ≤15 ml/min) or dialysis, hypercalcaemia (>2.6 mmol/l); hemiplegia		G5–G8: 10.2 Background calcium intake: NR Compliance Pill count method – at least 80% of total study pills taken (%) All: 85.8 Serum 25(OH)D at 3 y (ng/mL) G1–G4: 24.4 ng/mL [61 nmol/L] on average G5–G8: 37.6 ng/mL [94 nmol/L] on average.		≥ 1 fall, unadjusted OR (95% CI) G5–G8 vs G1–G4: 1.02 (0.85, 1.22) ≥ 1 fall, adjusted OR (95% CI) [4)] G5–G8 vs G1–G4: 1.02 (0.85, 1.23). **Injurious falls** Injurious falls over 3 y, *n* of events G1–G4: 1068 G5–G8: 1073 ≥1 injurious fall over 3 y, *n* of participants G1–G4: 548 G5–G8: 570 Injurious falls, unadjusted IRR (95% CI) G5–G8 vs G1–G4: 1.01 (0.90, 1.13) Injurious falls, adjusted IRR (95% CI) [4)] G5–G8 vs G1–G4: 1.03 (0.92, 1.14). ≥ 1 injurious fall, unadjusted OR (95% CI) G5–G8 vs G1–G4: 1.10 (0.92, 1.30) ≥ 1 injurious fall, adjusted OR (95% CI) [4)]	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	or other severe gait impairment; history of hypo- or primary hyperparathyroidism; severe liver disease; history of granulomatous diseases (i.e. tuberculosis, sarcoidosis); major visual or hearing impairment or other serious illness that would preclude participation; living with a partner who is enrolled in DO-HEALTH (i.e. only one person per household can be enrolled); living in assisted living situations or a nursing home; epilepsy and/or use of anti-epileptic drugs; individuals who fell more than 3 times in the last month; osteodystrophia deformans (M. Paget, Paget's disease); for study center in Germany only: persons who were institutionalized / in prison by court order. Temporary exclusion: acute fracture in the last 6 weeks. N participants randomised/ completed/analysed G1–G4: 1081/907/1081 G5–G8: 1076/938/1076				G5–G8 vs G1–G4: 1.11 (0.93, 1.33). 3) adjusted for age, sex, prior falls, BMI, and study site. 4) adjusted for study site, sex, age, previous fall, baseline BMI, and baseline use of walking aids.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
Burt et al. (2019) Canada Latitude [assumed] 51.05° N 3 y Private funding	RCT (parallel) Inclusion criteria: Healthy men and women aged 55–70 years; DXA lumbar spine and total hip areal BMD T score greater than −2.5 SD, serum 25 (OH)D between 30–125 nmol/L, and normal serum calcium (2.10–2.55 mmol/L). Participants were requested to take no more than 200 IU [5 μg] per day of additional vitamin D (eg. a multivitamin supplement). Exclusion criteria: Serum25(OH)D < 30 nmol/L or > 125 nmol/L; serum calcium >2.55 mmol/L or <2.10 mmol/L; vitamin D supplement use >2000 IU/d [50 μg/d] for the past 6 mo; use of bone active medication within the last 2 y; disorders known to affect vitamin D metabolism such as sarcoidosis, renal failure, malabsorption disorders, kidney stone within the past 2 y, or regular use of tanning salons; DXA T score	Sex: (% females) G1: 42.9 G2: 48.5 G3: 47.5 Age (y) G1: 62.2 ± 4.2 G2: 62.6 ± 4.3 G3: 61.9 ± 4.1 Years since menopause G1: 12.6 ± 5.9 G2: 11.7 ± 7.3 G3: 12.5 ± 5.6 Serum 25(OH)D (nmol/L) G1: 76.7 ± 21.0 G2: 81.3 ± 20.1 G3: 78.4 ± 18.4 BMI (kg/m^2) G1: 27.7 ± 4.3 G2: 28.1 ± 5.0 G3: 27.1 ± 4.1 Ethnicity (%) Non-Hispanic White: G1: 93.3 G2: 96.9 G3: 97.0 Non-Hispanic black: G1: 1.0 G2: 1.0 G3: 0 Asian:	Vitamin D_3 Doses: G1: vitamin D_3 400 IU/d [10 μg/d] G2: vitamin D_3 4000 IU/d [100 μg/d] G3: vitamin D_3 10 000 IU/d [250 μg/d] Participants not consuming the recommended dietary allowance of calcium (1200 mg/d) received calcium tablets as needed (≤maximum of 600 mg/d) to approximate a total daily intake of 1200 mg. Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – number of days of supplement administration vs total number of days (%) All: 99 on average, range (range 81–100). Serum 25(OH)D at 36 mo (nmol/L)	**Fractures** - reported as adverse events - reviewed by study clinicians via participant medical records and x-ray reports where available - fractures of fingers or toes and traumatic fractures were not included. Low-trauma (fragility) fractures: -reported as adverse events - defined as resulting from low trauma, such as a fall from standing height or less. **Falls** - reported as adverse events - adjudicated by the study physicians. **** Assay of serum 25 (OH)D: CLIA (DiaSorin Liaison XL system)	Data extracted: unclear **Low-trauma fractures** Low-trauma fractures over 3 y, *n* of events G1: 4 G2: 3 G3: 5 ≥ 1 low-trauma fracture over 3 y, *n* of participants G1: 4 G2: 3 G3: 5 **Falls** Falls over 3 y, *n* of events G1: 4 G2: 11 G3: 6 ≥ 1 fall over 3 y, *n* of participants G1: 4 G2: 10 G3: 5 Stated that no significant difference in falls detected among the 3 groups.	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	compatible with osteoporosis at the lumbar spine, total hip or femoral neck; and high 10-year risk (≥20%) for osteoporotic fracture (as defined by the World Health Organization's Canadian FRAX calculator). N participants, randomised/ completed/analysed: G1: 109/100/105 G2: 100/94/97 G3: 102/93/101	G1: 4.7 G2: 2.1 G3: 2.0 Hispanic: G1: 1.0 G2: 0 G3: 1.0 Smokers (%) G1: 1.9 G2: 0 G3: 4.0 Alcohol use: NR Health status: NR Fracture experienced after age 50 y (%) G1: 16.2 G2: 13.4 G3: 16.8 Falls in the last year (%) G1: 21.0 G2: 20.6 G3: 17.8 Season: Aug 2013 to Dec 2017.	G1: 77.4 ± 17.9 G2: 132.2 ± 28.0 G3: 144.4 ± 40.4			
Flicker et al. (2005) Australia Latitude 32° S, 34° S, 38° S	RCT (parallel) Inclusion criteria: Older people resident in hostels and nursing homes in urban and rural centers;	Sex (% females) G1: 95 G2: 95 Age (y) G1: 83.3 ± 8.8 G2: 83.6 ± 7.8	Vitamin D_2 Doses G1: placebo + calcium 600 mg/d G2: vitamin D_2 10 000 IU/wk [250 μg/	**Fractures** - verified using x-ray report when possible. **Falls** - defined as an event that results in a	Data extracted: ITT **Fractures** ≥ 1 fracture over 2 y, *n* of participants G1: 35 G2: 25	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
[study residents in three states] 2 y Mixed funding	25(OH)D level > 25 nmol/L and < 90 nmol/L. Exclusion criteria: 25(OH)D level < 25 nmol/L or > 90 nmol/L; use of agents that could affect bone and mineral metabolism, such as warfarin, chronic heparin therapy, vitamin D therapy within the previous 3 mo, glucocorticoids at an average daily dose of >5 mg prednisolone (or equivalent) for >1 mo within the preceding year; current use of bisphosphonates, and hormone replacement therapy; thyrotoxicosis within the previous 3 years, primary hyperparathyroidism treated within the previous 3 years, multiple myeloma, Paget's disease of bone, a history of malabsorption, intercurrent active malignancy, and other disorders affecting bone and mineral metabolism. N participants randomised/ completed/analysed	Serum 25(OH)D (%) 25–40 nmol/L: G1: 54 G2: 61 41–60 nmol/L: G1: 35 G2: 28 61–90 nmol/L: G1: 11 G2: 11 BMI: NR Ethnicity [assumed]: Majority Caucasian Smoking status: NR Alcohol use: NR Health status: NR Previous fracture (%) Hip: G1: 12 G2: 17 Colles: G1: 5 G2: 3 Recorded vertebral: G1: 6 G2: 4 Other: G1: 7 G2: 9 Any: G1: 24 G2: 27	wk = 35.71 μg/d] until Nov 1998; thereafter 1000 IU/d [=25 μg/d] [6] + calcium 600 mg/d. [6] due to the discontinuation of the preparation of commercial 10 000 IU [250 μg] vitamin D_2 tablets. Background vitamin D intake: NR Background calcium intake: NR Background calcium supplement use (%) G1: 5 G2: 4 Compliance Pill count method - vitamin D supplements removed from the participant's medication container (%) 0–25%: G1: 5 G2: 4 26–50%: G1: 7 G2: 7 51–75%: G1: 21	person coming to rest inadvertently on the ground or other lower level - recorded by residential care staff prospectively in diaries. **** Assay of serum 25 (OH)D: NR	≥ 1 fracture, OR (95% CI) G2 vs G1: 0.69 (0.40, 1.18) **Falls** Falls over 2 y, *n* of events G1: 890 G2: 665 ≥ 1 fall over 2 y, *n* of participants G1: 185 G2: 170 Falls, IRR (95% CI) G2 vs G1: 0.73 (0.57, 0.95) ≥ 1 fall, OR (95% CI) G2 vs G1: 0.82 (0.59, 1.12) Data extracted: PP [5] **Fractures** ≥ 1 fracture over 2 y, *n* of participants G1: 30 G2: 21 ≥ 1 fracture, OR (95% CI) G2 vs G1: 0.68 (0.38, 1.22) **Falls** Falls over 2 y, *n* of events G1: 862 G2: 570 ≥ 1 fall over 2 y, *n* of participants G1: 176	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	G1: 312/unclear/ITT 312, PP 271 [5)] G2: 313/unclear/ITT 313, PP 269 [5)] [5)] those whose vitamin D compliance was ≥50%	Season: NR	G2: 18 76–100%: G1: 66 G2: 68 Unknown: G1: 1 G2: 3 Serum 25(OH)D at 2 yr: NR		G2: 152 Falls, IRR (95% CI) G2 vs G1: 0.63 (0.48, 0.82) ≥ 1 fall, OR (95% CI) G2 vs G1: 0.70 (0.50, 0.99) [5)] those whose vitamin D compliance was ≥50%	
Grant et al. (2005) **RECORD** United Kingdom Latitude [assumed] 51° N to 58° N; the trial was based in 21 hospitals in United Kingdom 24 to 62 mo (median 45, IQR 37–52) Mixed funding	RCT (parallel) Inclusion criteria: People aged ≥70 years who had had a low trauma, osteoporotic fracture in the previous 10 years. Exclusion criteria: Bed or chair bound before fracture; cognitive impairment indicated by an abbreviated mental test score of <7; cancer in the past 10 years that was likely to metastasise to bone; fracture associated with pre-existing local bone abnormality; those known to have hypercalcaemia; renal stone in the past 10 years; life expectancy of <6 mo; individuals known	Sex (% females) G1–G2: 85 G3–G4: 85 Age (y) G1–G2: 77 ± 6 G3–G4: 77 ± 6 Serum 25(OH)D (ng/mL) All: 15.2 ± 6.5 ng/mL [38 ± 16.25 nmol/L] NR across groups. BMI: NR Ethnicity (%) White: G1–G2: 99 G3–G4: 99 Smoking status (%) Current smokers: G1–G2: 12.1	Vitamin D_3 Doses G1: double placebo G2: vitamin D placebo + calcium 1000 mg/d G3: vitamin D_3 800 IU/d [20 μg/d] + calcium placebo G4: vitamin D_3 800 IU/d [20 μg/d] + calcium 1000 mg/d Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – taking tablets >80% of days at 24 mo (%):	**Fractures** - clinical, radiological confirmed vertebral fractures excluding those of the face or skull - were obtained from postal questionnaires every 4 mo with telephone follow-up if needed, hospital and general practice staff, nominated friends or relatives of participants, and national routine data-collection systems of the UK Office of National Statistics, the Information and Statistics Division (Scotland), and the	Data extracted: ITT **New fractures** New fractures over the intervention, *n* of participants G1–G2: 385 G3–G4: 396 New fractures, HR (95% CI) G3–G4 vs G1–G2: 1.01 (0.88, 1.17) **Confirmed fractures** Confirmed fractures over the intervention, *n* of participants G1–G2: 377 G3–G4: 387 Confirmed fractures, HR (95% CI) G3–G4 vs G1–G2: 1.01 (0.87, 1.16)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	to be leaving the UK; daily intake of >200 IU [5 μg/d] vitamin D or > 500 mg calcium supplements; intake in the past 5 years of fluoride, bisphosphonates, calcitonin, tibolone, hormone replacement therapy, selective oestrogen-receptor modulators, or any vitamin D metabolite (eg, calcitriol); and vitamin D by injection in the past year. N participants randomised/ completed/analysed G1–G2: 2643/2382 at 24 mo, 572 at 48 mo, 29 at 60 mo /2382 at 24 mo, 572 at 48 mo, 29 at 60 mo G3–G4: 2649/2401 at 24 mo, 582 at 48 mo, 24 at 60 mo /2401 at 24 mo, 582 at 48 mo, 24 at 60 mo	G3–G4: 11.3 Alcohol use: NR Health status: A history of low-trauma, osteoporotic fracture in the previous 10 years. An osteoporotic fracture was defined as a fracture due to a fall from no more than standing height, or as a definite clinical event with radiologist-confirmed evidence of a vertebral fracture. Previous fracture since age 50 y (%) G1: 35.3 G2: 35.0 Season: Feb 1999 to March 2002.	G1–G2: 78.8 G3–G4: 78.4 Completing questionnaires >80% of days at 24 mo (%): G1–G2: 59.9 G3–G4: 60.6 Serum 25(OH)D, change over 1 yr (ng/mL) G1: +3.1 ± 7.2 ng/mL [7.75 ± 18 nmol/L] G2: +1.4 ± 5.7 ng/mL [3.5 ± 14.25 nmol/L] G3: +9.7 ± 8.7 ng/mL [24.25 ± 21.75 nmol/L] G4: +9.6 ± 6.9 ng/mL [24.0 ± 17.25 nmol/L]	Hospital Episode Statistics gathered by the Department of Health in England - confirmation always sought from a second source. **Falls** - defined by means of the question" Have you fallen during the last week?" - obtained from the postal questionnaires and were supplemented by further clinical information on potentially serious adverse events. **** Assay of serum 25 (OH)D: HPLC	**Low-trauma fractures** Low-trauma fractures over the intervention, *n* of participants G1–G2: 345 G3–G4: 353 Low-trauma fractures, HR (95% CI) G3–G4 vs G1–G2: 1.02 (0.88, 1.19) **Falls** Falls over the intervention, *n* of participants G1–G2: 381 G3–G4: 380 Falls, HR (95% CI) G3–G4 vs G1–G2: 0.97 (0.84, 1.12)	
Hin et al. (2016) **BEST-D** United Kingdom Latitude [assumed] 51° N to 58° N 12 mo	RCT (parallel) Inclusion criteria: Ambulatory, aged 65 y or more, living in the community, not currently taking more than 10 μg/d vitamin D_3.	Sex (% females) G1: 49 G2: 50 G3: 49 Age (y) G1: 72 ± 6 G2: 72 ± 6	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 2000 IU/d [50 μg/d] G3: vitamin D_3 4000 IU/d [100 μg/d]	**Fractures** - self-reported - measured at all sites and specific sites - recorded at 6 and 12 mo - no additional information provided.	Data extracted: ITT **Any fracture**, *n* of participants G1: 1 G2–G3: 6 *P* = 0.31 for difference in numbers between groups.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
Mixed funding	Exclusion criteria: Nursing home residents; regular users of vitamin D supplements more than 10 μg/d; prescribed calcium supplements, bisphosphonates, parathyroid hormone (PTH), or calcitonin; had medically diagnosed dementia or history of hypercalcaemia, hyperparathyroidism, lymphoma, sarcoidosis, active tuberculosis or renal calculus; judged by own doctor as likely to be poorly compliant with clinic visits or medication; or a history of alcohol or substance misuse or a history that might limit the ability to take the study treatment (e.g. terminal illness). N participants randomised/ completed/analysed G1: 101/95/101 G2: 102/98/102 G3: 102/97/102	G3: 71 ± 6 Serum 25(OH)D (nmol/L) G1: 47 ± 1.5 G2: 55 ± 2.2 G3: 49 ± 1.5 BMI (kg/m^2) G1: 28 ± 5 G2: 27 ± 4 G3: 27 ± 5 Ethnicity: White Smoking status (%) Current smokers: G1: 7 G2: 7 G3: 7 Alcohol use: NR Health status (%) Hypertension: G1: 35 G2: 43 G3: 39 Diabetes: G1: 9 G2: 9 G3: 9 Fracture experienced ever (%) G1: 30 G2: 29 G3: 30	Background vitamin D intake: NR Background vitamin D supplement use ≤10 μg/d (%) G1: 13 G2: 10 G3: 12 Background calcium intake (mg/d) G1: 713 ± 302 G2: 695 ± 292 G3: 724 ± 287 Background calcium supplement use (%) G1: 4 G2: 1 G3: 4 Compliance Pill count method – capsules taken on all or most days (%) At 6 mo: G1: 87 G2: 93 G3: 93 At 12 mo: G1: 85 G2: 92 G3: 90	**Falls** - self-reported - recorded at 6 and 12 mo - no additional information provided. **** Assay of serum 25 (OH)D: CLIA (Beckman Coulter Ltd., High Wycombe, England).	**Any fall**, *n* of participants G1: 14 G2–G3: 34 *P* = 0.53 for difference in numbers between groups.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
		Any fall in the past 6 mo (%) G1: 12 G2: 15 G3: 13 Season: Randomisation occurred between Sept 2012 and March 2013.	Serum 25(OH)D at 12 mo (nmol/L) G1: 53 ± 2.4 G2: 102 ± 2.4 G3: 137 ± 2.4			
LeBoff et al. (2020a) **VITAL** USA Latitude [assumed] 19° N to 64.9° N; this was a nationwide study 5 y, (median 5.3, range 3.8–6.1) Mixed funding	RCT (parallel) Inclusion criteria: Men age ≥ 50 and women age ≥ 55 years. Exclusion criteria: A history of cancer or cardiovascular disease; cirrhosis, hypercalcaemia, renal failure or dialysis, and other serious conditions. Personal use of vitamin D_3 was limited to 800 IU/d [20 μg/d] or less and 1200 mg/day or less for calcium. N participants randomised/ completed/analysed G1–G2: 12944/NR/12867 at baseline, 12119 at year 1, 11861 at year 2, 11334 at year 3, 10764 at year 4, 9960 at year 5	Sex (% females) G1–G2: 50.5 G3–G4: 50.7 Age (y) G1–G2: 67.14 ± 7.08 G3–G4: 67.13 ± 7.05 Serum 25(OH)D (nmol/L) G1–G2: 76.6 ± 25 G3–G4: 76.8 ± 25 BMI (kg/m^2) G1–G2: 28.07 ± 5.79 G3–G4: 28.12 ± 5.68 Ethnicity (%) Non-hispanic white: G1–G2: 71.4 G3–G4: 71.3 Black: G1–G2: 20.2 G3–G4: 20.2 Non-black Hispanic:	Vitamin D_3 Doses G1: double placebo G2: vitamin D placebo + omega-3 fatty acids 1 g/d G3: vitamin D_3 2000 IU/d [50 μg/d] + omega-3 fatty acid placebo G4: vitamin D_3 2000 IU/d [50 μg/d] + omega-3 fatty acids 1 g/d Background dietary vitamin D intake: NR Background vitamin D intake ≤800 IU/d [20 μg/d] (%): G1–G2: 42.8 G3–G4: 42.5 Background dietary calcium intake: NR Background calcium intake ≤1200 mg/d (%):	**Falls** - defined as unintentionally coming to rest on the ground, floor, or lower surface - numbers were recorded in questionnaires by the participants. **Injurious falls** - defined as those resulting in limited regular activity for ≥1 day or in a doctor visit. **Falls requiring hospitalization** or being evaluated by a health care provider - yes/no ≥ 2 falls was selected as the main outcome. Single falls without	Data extracted: ITT **Falls** ≥ 2 falls, *n/N* Year 1 G1–G2: 1059/12119 G3–G4: 1075/12168 Year 2 G1–G2: 1158/11861 G3–G4: 1167/11879 Year 3 G1–G2: 1130/11334 G3–G4: 1155/11410 Year 4 G1–G2: 1048/10764 G3–G4: 1102/10914 Year 5 G1–G2: 1127/9960 G3–G4: 1202/10099 ≥ 2 falls, OR (95% CI), average proportions per year over 5 years G3–G4 vs G1–G2: 0.97 (0.90, 1.05)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	G3–G4: 12927/NR/12848 at baseline, 12168 at year 1, 11879 at year 2, 11410 at year 3, 10914 at year 4, 10099 at year 5	G1–G2: 3.9 G3–G4: 4.08 Asian: G1–G2: 1.6 G3–G4: 1.5 Native American or Alaskan native: G1–G2: 0.87 G3–G4: 0.92 Other or unknown: G1–G2: 2.09 G3–G4: 2.05 Smoking status (%) Current smokers: G1–G2: 7.2 G3–G4: 7.2 Alcohol use (%) Never: G1–G2: 31.6 G3–G4: 31.3 Rarely to weekly: G1–G2: 7.6 G3–G4: 7.4 1-6/wk: G1–G2: 34.4 G3–G4: 35.5 Daily: G1–G2: 26.4 G3–G4: 25.8 Health status (%) "In general would you say your health is…" Excellent:	G1–G2: 19.6 G3–G4: 20.3 <u>Compliance</u> Pill count method – those taking ≥2/3 of the trial capsules (%) Among those answering a compliance question by questionnaire: G1–G2: 84.7 G3–G4: 85.9 Among all participants including nonrespondents to questionnaires: G1–G2: 74. G3–G4: 76.1 Serum 25(OH)D at 1 yr (nmol/L) G1–G2: NR G3–G4: 104 on average	injury was not included. **** Assay of serum 25 (OH)D: LC–MS/MS	**Injurious falls** ≥ 1 injurious fall, *n/N* Year 1 G1–G2: 823/12119 G3–G4: 811/12168 Year 2 G1–G2: 855/11861 G3–G4: 831/11879 Year 3 G1–G2: 814/11334 G3–G4: 877/11410 Year 4 G1–G2: 788/10764 G3–G4: 818/10914 Year 5 G1–G2: 945/9960 G3–G4: 1005/10099 ≥ 1 fall resulting in injury, OR (95% CI), average proportions per year over 5 years G3–G4 vs G1–G2: 1.03 (0.94, 1.13) **Falls requiring hospitalization** ≥ 1 fall requiring hospitalization, *n/N* Year 1 G1–G2: 588/12119 G3–G4: 544/12168 Year 2 G1–G2: 588/11861 G3–G4: 566/11879 Year 3	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
		G1–G2: 28.9 G3–G4: 29.3 Very good: G1–G2: 45.0 G3–G4: 44.5 Good: G1–G2: 22.5 G3–G4: 22.3 Fair: G1–G2: 3.4 G3–G4: 3.8 Poor: G1–G2: 0.27 G3–G4: 0.22 History of fragility fracture (%) G1: 9.97 G2: 9.96 Fallen in prior year (%) G1: 32.6 G2: 33.8 Season: NR			G1–G2: 550/11334 G3–G4: 595/11410 Year 4 G1–G2: 554/10764 G3–G4: 570/10914 Year 5 G1–G2: 666/9960 G3–G4: 732/10099 ≥ 1 fall resulting in hospital, OR (95% CI), average proportions per year over 5 years G3–G4 vs G1–G2: 1.04 (0.90, 1.19)	
Macdonald et al. (2013) United Kingdom Latitude 57° N 1 yr Mixed funding	RCT (parallel) Inclusion criteria: Healthy postmenopausal, non-smoking women aged 60–70 years; not suffering from any condition (diabetes, asthma, malabsorption, blood pressure > 160 mmHg	Sex: Females Age (y) G1: 64.6 ± 2.3 G2: 64.2 ± 1.9 G3: 64.9 ± 2.2 Serum 25(OH)D (nmol/L) G1: 35.8 ± 16.4 G2: 33.4 ± 13.2	Vitamin D_3 Doses [labeled] G1: placebo G2: vitamin D_3 400 IU/d [10 μg/d] G3: vitamin D_3 1000 IU/d [25 μg/d] Analysed doses G2: 346 IU [8.65 μg/d]	**Fractures** - reported as adverse events. **** Assay of serum 25 (OH)D: LC–MS/MS	Data extracted: unclear ≥ 1 fracture, *n* of participants G1: 3 G2: 3 G3: 0 There were 7 fractures during the study, with one	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	systolic or > 99 mmHg diastolic); not taking medication (hypotensive, hypolipemic, ant-inflammatory, oral corticosteroid) likely to affect vitamin D metabolism or cardiovascular disease risk. Women on thyroxine treatment were included if stable, as assessed by free T4 and thyroid stimulating hormone concentrations, and their dose had not changed in the 3 mo before study entry. Exclusion criteria: Planned frequent trips or long periods abroad that would result in an increased exposure to UVB light, or an abnormal biochemical profile on screening. N participants randomised/ completed/analysed G1: 102/90/ITT 90, PP 88 G2: 102/85/ITT 84, PP 84 G3: 101/90/ITT 90, PP 88	G3: 33.2 ± 13.8 BMI (kg/m^2) G1: 25.9 ± 3.8 G2: 25.3 ± 3.9 G3: 25.2 ± 3.4 Ethnicity: White Smoking status: Non-smokers Alcohol use: NR Health status: Healthy Season: Jan–March at baseline.	G3: 832 IU [20.8 μg/d] Background vitamin D intake (μg/d) G1: 5.6 ± 3.0 G2: 4.6 ± 2.5 G3: 5.3 ± 2.9 Background calcium intake (mg/d) G1: 1291 ± 492 G2: 1261 ± 488 G3: 1306 ± 568 Compliance Pill count method (%) All: 92 on average (range 72–98) Serum 25(OH)D at 1 yr (nmol/L) G1: 32.0 ± 1.6 G2: 65.0 ± 2.1 G3: 76.0 ± 2.0 reported as mean ± SEM		woman having two fractures on two separate occasions: - wrist/lower arm, *n* = 3 - foot/ankle, *n* = 3 - clavicle, *n* = 1	
Peacock et al. (2000) USA, Indiana	RCT (parallel) Inclusion criteria: Women and men aged 60 and over.	Sex (% females) G1: 73 G2: 71 G3: 72	Calcidiol Doses [labeled] G1: placebo G2: calcium 750 mg/d	**Fractures** - vertebral fractures: defined as a reduction of the anterior	Data extracted: unclear **Fractures** Nonvertebral fractures over 4 y, *n* of events	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
Latitude [assumed] 42.74° N 4 y Public funding	Exclusion criteria: A terminal illness; Paget's disease of bone; recurrent urinary stone disease; had been treated with sodium fluoride, bisphosphonate, steroids, or dilantin; renal disease requiring specific treatment; or excluded by their primary physician. N participants Randomised: All: 437 Completed: All: 236 Baseline information available[7)]: Females G1: 98 G2: 89 G3: 95 Male G1: 37 G2: 37 G3: 37 [7)] those who had measurement of bone mineral density at femoral neck, total hip, or lumbar spine and were studied at least one visit after baseline.	Age (y) Females G1: 72.3 ± 7.5 G2: 73.9 ± 8.0 G3: 74.1 ± 8.3 Male G1: 75.4 ± 7.6 G2: 76.0 ± 7.7 G3: 75.5 ± 7.2 Serum 25(OH)D (nmol/L) Females G1: 60.0 ± 30 G2: 62.5 ± 25 G3: 57.5 ± 33 Male G1: 65.0 ± 30 G2: 67.5 ± 23 G3: 65.0 ± 25 BMI: NR Ethnicity: White Smoking status: NR Alcohol use: NR Health status: NR Season: NR	G3: calcidiol 15 µg/d Analysed doses: Capsules analysed every 6 mo; no significant decrease observed over 4 y. Background vitamin D intake: NR Background calcium intake (mg/d) Females G1: 586 ± 290 G2: 564 ± 294 G3: 572 ± 267 Male G1: 629 ± 249 G2: 670 ± 325 G3: 739 ± 335 <u>Compliance</u> Pill count method – calcidiol capsules at each visit (%) All: 89 ± 16 Pill count method – calcium tablets at each visit (%) All: 80 ± 20 Pill count method – placebo capsules at each visit (%): All: 85 ± 19 Serum 25(OH)D at the endpoint: NR	vertebral height of 20% or greater - obtained from diaries kept by the participants - were recorded at each visit along with the type of trauma responsible, whether the fracture was confirmed by radiography, and whether it required splinting or surgery. - the occurrence of vertebral fractures from L4 to T4 was assessed from lateral thoracic and lumbar radiographs taken at the first and last visit for each subject. **** Assay of serum 25 (OH)D: CPBA (vitamin D-binding protein from rat serum) after HPLC	Females G1: 9 G2: 9 G3: 10 Male G1: 1 G2: 2 G3: 4 Vertebral fractures over 4 y, *n* of events Females G1: 10 G2: 5 G3: 15 Male G1: 3 G2: 2 G3: 4 Total fractures over 4 y, *n* of events Females G1: 19 G2: 14 G3: 25 Male G1: 4 G2: 4 G3: 8	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
Prince et al. (2008) Australia Latitude 32° S 1 yr Public funding	RCT (parallel) Inclusion criteria: Women aged 70–90 years, with a history of falling in the past 12 months with a plasma 25(OH)D concentration < 24.0 ng/mL [< 60 nmol/L]. Exclusion criteria: Current vitamin D consumption; current consumption of bone or mineral active agents apart from calcium; a bone mineral density z score at the total hip site of less than −2.0; medical conditions or disorders that influence bone mineral metabolism, including laboratory evidence of renal insufficiency (a creatinine level more than 2-fold above the reference range); a fracture in the past 6 months; a Mini-Mental State Examination score of <24; or the presence of marked neurological conditions likely to substantially impair balance or physical activity, such as stroke and Parkinson disease.	Sex: Females Age (y) G1: 77.4 ± 5.0 G2: 77.0 ± 4.2 Serum 25(OH)D (ng/mL) G1: 17.7 ± 5.1 ng/mL [44.3 ± 12.8 nmol/L] G2: 18.1 ± 5.0 ng/mL [45.3 ± 12.5 nmol/L] BMI: NR Ethnicity: NR Smoking status: NR Alcohol use: NR Health status: NR Number of falls in the past 12 mo (%) 1: G1: 57.6 G2: 59.6 2: G1: 26.5 G2: 27.2 3: G1: 13.2 G2: 9.9 > 3: G1: 2.6 G2: 3.3	Vitamin D_2 Doses G1: placebo + calcium 1000 mg/d G2: vitamin D_2 1000 IU/d [25 μg/d] + calcium 1000 mg/d. Background vitamin D intake: NR Background calcium intake (mg/d) G1: 1080 ± 426 G2: 1067 ± 484 Compliance Pill count method (%) G1: 86 G2: 86 Serum 25(OH)D in winter/spring G2 vs. G1: 28.1% higher. Serum 25(OH)D in summer/autumn G2 vs. G1: 12.5% higher.	**Fractures** - reported as adverse events. **Falls** - defined as unintentionally coming to rest on the ground, floor, or other lower level - obtained by interviewing participants every 6 wk; the number of falls that had occurred in the previous 6 weeks and the associated features of the falls were recorded on a falls questionnaire. **** Assay of serum 25 (OH)D: RIA (Dia-Sorin, Stillwater, Minnesota)	Data extracted: ITT **Fractures** Fractures over 1 yr, rate (%) G1: 2.0 G2: 2.6 **Falls** ≥ 1 fall, *n* of participants G1: 95 G2: 80 1 fall, OR (95% CI) G2 vs G1: 0.50 (0.28, 0.88) ≥ 1 fall, OR (95% CI) G2 vs G1: 0.66 (0.41, 1.06) ≥ 1 fall adjusted for baseline height, OR (95% CI) G2 vs G1: 0.61 (0.37, 0.99) ≥ 2 falls, OR (95% CI) G2 vs G1: 0.86 (0.50, 1.49) First fall in summer/autumn, OR (95% CI) G2 vs G1: 0.81 (0.46, 1.42) First fall in winter/spring, OR (95% CI) G2 vs G1: 0.55 (0.32, 0.96) First fall in winter/spring, RR (95% CI) G2 vs G1: 0.77 (0.56, 0.98)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	N participants randomised/ completed/analysed G1: 151/131/ ITT 151 G2: 151/124/ ITT 151	Season: Recruitment from Apr 2003 to Oct 2004.				
Smith et al. (2017) (Earlier publication by Gallagher et al. 2012 was used for baseline information) **ViDOS** USA Latitude 41° N 1 yr Mixed funding	RCT (parallel) Inclusion criteria: Healthy, white, postmenopausal women aged 57 to 90 years who were at least 7 years postmenopausal (determined from the history of their last menstrual period) with vitamin D insufficiency Exclusion criteria: Significant health problems, active nephrolithiasis or history of more than two kidney stones in their lifetime, chronic renal failure (serum creatinine >1.4 mg/dL), chronic liver disease, medical conditions severe enough to prevent reasonable physical activity, serum 25(OH)D levels <5 ng/mL (12.5 nmol/L), serum calcium levels of 10.3 mg/dL (2.575 mmol/L) or more or serum calcium levels more than 0.3 mg/dL higher than the upper	Sex: Females Age (y) G1: 66 ± 6.5 G2: 68 ± 8.6 G3: 68 ± 8.1 G4: 66 ± 7.4 G5: 66 ± 6.3 G6: 69 ± 7.7 G7: 66 ± 7.1 G8: 65 ± 6.1 Serum 25(OH)D (nmol/L) G1: 37.7 ± 9.1 G2: 37.8 ± 10.8 G3: 39.0 ± 9.5 G4: 37.4 ± 10.2 G5: 38.2 ± 10.1 G6: 39.8 ± 8.2 G7: 37.2 ± 9.2 G8: 38.6 ± 9.1 BMI (kg/m^2) G1: 31.1 ± 5.2 G2: 30.3 ± 5.4 G3: 28.2 ± 6.1 G4: 30.0 ± 5.4 G5: 30.4 ± 5.4 G6: 30.2 ± 5.7 G7: 29.7 ± 6.4 G8: 32.1 ± 6.2	Vitamin D_3 Doses G1: placebo + calcium G2: vitamin D_3 400 IU/d [10 μg/d] + calcium G3: vitamin D_3 800 IU/d [20 μg/d] + calcium G4: vitamin D_3 1600 IU/d [40 μg/d] + calcium G5: vitamin D_3 2400 IU/d [60 μg/d] + calcium G6: vitamin D_3 3200 IU/d [80 μg/d] + calcium G7: vitamin D_3 4000 IU/d [100 μg/d] + calcium G8: vitamin D_3 4800 IU/d [120 μg/d] + calcium Calcium to maintain total intake between 1200 to 1400 mg/d (was based on a baseline 7-day food diary) Background vitamin D intake (IU/d) G1: 105 ± 61 IU/d [2.6 ± 1.5 μg/d] G2: 98 ± 58 IU/d [2.5 ± 1.5 μg/d]	**Falls** - defined as a sudden unintentional change in position causing an individual to land on the ground, floor, or at a lower level, with or without injury - details on the number of falls and whether a fall resulted in a break or fracture were collected by an interviewer every 3 mo. **** Assay of serum 25 (OH)D: RIA (Diasorin kit) and LC–MS	Data extracted: unclear ≥ 1 fall over 1 yr, rate (%) G1: 58 G2–G3: 53 G4–G6: 30 G7–G8: 69 *P* = 0.030 for difference in rates between groups. Lower faller rate in G4–G6 vs the other groups, *P* < 0.05. Falls over 1 yr, mean ± SE [8)] G1: 0.94 ± 0.23 G2–G3: 0.85 ± 0.19 G4–G6: 0.41 ± 0.09 G7–G8: 0.79 ± 0.19 *P* = 0.030 for difference between groups. In pairwise comparisons after adjusting for multiple comparisons fewer falls in G4–G6 vs G1, *P* = 0.058; G4–G6 vs G2–G3, *P* = 0.058; and G4–G6 vs G7–G8, *P* = 0.094. ≥ 1 fall, OR (95% CI) [9)] G1 vs G4–G6: 3.86 (1.24, 12.04)	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	normal limit on two baseline tests, and 24-h urine calcium levels >300 mg/dL (7.5 mmol) on two baseline tests; taking currently bisphosphonates or had taken them for >3 months in the past were excluded; use of fluoride, parathyroid hormone (PTH) or its derivatives, calcitonin, estrogen (in the last 6 mo), corticosteroids (>10 mg/d), phenytoin or phenobarbital, or high-dose thiazide (>37.5 mg/d); multivitamins containing vitamin D were not allowed in the study. N participants, randomised/completed/ analysed G1: 21/18/19 G2: 20/16/18 G3: 21/17/18 G4: 20/19/19 G5: 21/20/19 G6: 20/18/18 G7: 20/16/17 G8: 20/18/18 [Number of completed participants and their baseline characteristics obtained from the earlier	Ethnicity: White Smoking status (%) Current: G1: 19 G2: 10 G3: 5 G4: 20 G5: 5 G6: 15 G7: 0 G8: 10 Former: G1: 33 G2: 35 G3: 33 G4: 40 G5: 38 G6: 20 G7: 50 G8: 45 Never: G1: 48 G2: 55 G3: 62 G4: 40 G5: 57 G6: 65 G7: 50 G8: 45 Alcohol users (%) G1: 33 G2: 35 G3: 57 G4: 50	G3: 135 ± 70 IU/d [3.4 ± 1.8 μg/d] G4: 125 ± 71 IU/d [3.1 ± 1.8 μg/d] G5: 98 ± 55 IU/d [2.5 ± 1.4 μg/d] G6: 109 ± 62 IU/d [2.7 ± 1.6 μg/d] G7: 106 ± 83 IU/d [2.7 ± 2.1 μg/d] G8: 137 ± 86 IU/d [3.4 ± 2.2 μg/d] Background calcium intake (mg/d) G1: 593 ± 182 G2: 606 ± 212 G3: 741 ± 247 G4: 754 ± 244 G5: 612 ± 190 G6: 725 ± 263 G7: 673 ± 324 G8: 768 ± 348 Compliance Pill count method - vitamin D_3 capsules (%) All: 94 on average Pill count method – calcium tablets (%) All: 91 on average Serum 25(OH)D at 1 yr: G1 vs. the other groups: levels were significantly		G2–G3 vs G4–G6: 3.15 (1.24, 7.99) G7–G8 vs G4 –G6: 5.63 (2.14, 14.85) 8) predicted means; adjusted for age, BMI, smoking status and alcohol use. 9) adjusted for age, BMI, smoking status, alcohol use, and history of falls in the past 12 mo prior to the study. At 1 yr, the quadratic trend observed in faller rate was stronger for the quintiles of serum 25(OH)D concentrations measured by LC–MS than for those measured by RIA. By RIA, the highest faller rate of 72% was in the 4th quintile [range 38–45.9 ng/mL or 95–114.8 nmol/L]. By LC–MS, the highest faller rate of 68% was in the 1st quintile [<28 ng/mL or < 70 nmol/L].	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	publication the study referred to (Gallagher et al. 2012)]	G5: 52 G6: 70 G7: 70 G8: 80 Health status: Healthy. Fallen in the previous 12 mo (%) Caucasian: 32.2 African American: 25.3 Season: Enrolment in winter and spring over 2 years: Apr to May 2007 and Jan to May 2008.	lower (visual presentation).			
Uusi-Rasi et al. (2015) Finland Latitude [assumed] 61.5° N 2 y Mixed funding	RCT (parallel) Inclusion criteria: Home-dwelling women 70–80 years, fallen at least once during the previous 12 mo, did not use vitamin D supplements, and had no contraindications to exercise. Exclusion criteria: Participating in moderate to vigorous exercise >2 h/wk; regular use of vitamin D or	Sex: Females Age (y) G1: 73.8 ± 3.1 G2: 74.8 ± 2.9 G3: 74.1 ± 3.0 G4: 74.1 ± 2.9 Serum 25(OH)D (ng/mL) G1: 27.1 ± 7.5 ng/mL [67.8 ± 18.8 nmol/L] G2: 27.8 ± 7.2 ng/mL [69.5 ± 18.0 nmol/L]	Vitamin D_3 Doses G1: placebo + no exercise G2: placebo + exercise G3: vitamin D_3 800 IU/d [20 μg/d] + no exercise G4: vitamin D_3 800 IU/d [20 μg/d] + exercise Exercise = supervised, progressive group training classes 2 times/wk for the	**Falls** - defined as an unexpected event in which the participant comes to rest on the ground, floor or lower level - obtained from prospective diaries that were returned monthly via mail; details of each fall ascertained by a phone call.	Data extracted: ITT **Falls** All falls, rate per 100 Person-years G1: 118.2 G2: 120.7 G3: 132.1 G4: 113.1 All falls, IRR (95% CI) G2 vs G1: 1.07 (0.77, 1.45) G3 vs G1: 1.08 (0.78, 1.52) G4 vs G1: 0.99 (0.72, 1.39) Fallers, HR (95% CI)	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
	calcium + vitamin D supplements; a recent fracture (during preceding 12 months); a marked decline in the basic activities of daily living (ADL); cognitive impairments (Mini Mental State Examination, MMSE-test); primary hyperthyroidism; and degenerative conditions, such as Parkinson's disease. N participants randomised/ completed/analysed G1: 102/95/102 G2: 103/91/103 G3: 102/88/102 G4: 102/96/102	G3: 26.4 ± 6.9 ng/mL [66.0 ± 17.3 nmol/L] G4: 26.2 ± 7.0 ng/mL [65.5 ± 17.5 nmol/L] BMI: NR Ethnicity [assumed]: Majority Caucasian Smoking status: 13 women were current smokers. Alcohol use: Stated to be low. Health status (%) Hypertension: G1: 41 G2: 35 G3: 51 G4: 52 Cardiovascular disease: G1: 18 G2: 20 G3: 16 G4: 16 Hypothyroidism: G1: 19 G2: 24 G3: 17 G4: 26 Diabetes mellitus: G1: 8.8 G2: 9.7 G3: 12 G4: 5.8	first 12 mo and once/wk for the remaining 12 mo. Background vitamin D intake (μg/d) G1: 10.2 ± 4.1 G2: 10.3 ± 3.6 G3: 10.9 ± 4.2 G4: 10.4 ± 3.9 Background calcium intake (mg/d) G1: 1040 ± 345 G2: 1119 ± 346 G3: 1125 ± 420 G4: 1109 ± 385 Compliance Pill count method (%): All: 98.1 on average (range 42.6–100) Attendance at all offered training sessions (%): All: 72.8 on average (range 0–97.4) Serum 25(OH)D at 24 mo (ng/mL): G1–G2: 27.5 ± 6.9 ng/mL [68.8 ± 17.3 nmol/L] G3–G4: 37.0 ± 7.4 ng/mL [92.5 ± 18.5 nmol/L]	**Injurious falls** - defined as those for which participants sought medical care; included e.g. bruises or fractures. **** Assay of serum 25 (OH)D: EIA (Octeia; IDS)	G2 vs G1: 0.93 (0.66, 1.31) G3 vs G1: 0.77 (0.54, 1.11) G4 vs G1: 0.91 (0.64, 1.28) Multiple fallers, HR (95% CI) G2 vs G1: 1.14 (0.76, 1.71) G3 vs G1: 1.07 (0.71, 1.62) G4 vs G1: 1.14 (0.77, 1.71) **Injurious falls** Injurious falls, rate per 100 Person-years G1: 13.2 G2: 6.5 G3: 12.9 G4: 5.0 Injurious falls, IRR (95% CI) G2 vs G1: 0.46 (0.22, 0.95) G3 vs G1: 0.84 (0.45, 1.57) G4 vs G1: 0.38 (0.17, 0.81) Injured fallers, HR (95% CI) G2 vs G1: 0.47 (0.23, 0.99) G3 vs G1: 0.89 (0.47, 1.69) G4 vs G1: 0.38 (0.17, 0.83) Cumulative hazard presented.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
		Osteoarthritis: G1: 23 G2: 25 G3: 28 G4: 33 Depression: G1: 2.0 G2: 1.9 G3: 4.9 G4: 4.9 Season: April 2010 to March 2013.				
Wood et al. (2014) United Kingdom Latitude 57° N 1 yr Mixed funding	RCT (parallel) Inclusion criteria: Healthy postmenopausal women aged 60–70 years, BMI 18–45 kg/m^2. Exclusion criteria: Severe disease; taking vascular medications or dietary supplements known to contain vitamin D_2 or D_3; current smokers; participants with abnormal blood biochemistry at study screening. N participants randomised/ completed/analysed G1: 102/91/100 G2: 102/84/97 G3: 101/90/96	Sex: Females Age (y) G1: 63.9 ± 2.3 G2: 63.5 ± 1.9 G3: 64.1 ± 2.3 Serum 25(OH)D (nmol/L) G1: 36.18 ± 17.1 G2: 32.74 ± 12.9 G3: 32.41 ± 13.8 BMI G1: 26.6 ± 4.4 G2: 26.6 ± 4.2 G3: 26.8 ± 4.2 Ethnicity: Caucasian Smoking status: Non-smokers. Alcohol use: NR	Vitamin D_3 Doses [labeled] G1: placebo G2: vitamin D_3 400 IU/d [10 μg/d] G3: vitamin D_3 1000 IU/d [25 μg/d] Analysed doses G2: 346 IU [8.65 μg] G3: 832 IU [20.8 μg] Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method (%) All: >95% Serum 25(OH)D, change over 12 mo (nmol/L),	Falls - not defined - recorded at each study visit by asking the question" Have you had any recent falls in the past 2 months?" **** Assay of serum 25 (OH)D: MS/MS	Data extracted: ITT Falls over 12 mo, *n* of events G1: 40 G2: 48 G3: 30 ≥1 fall over 12 mo, *n* of participants G1: 31 G2: 33 G3: 27 *P* = 0.65 for difference in incidence between groups.	**2**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed Assay of serum 25(OH)D	Results	RoB Tier
		Health status: Relatively healthy. Fallen over the last 2 mo prior to the study (%) G1: 7 G2: 8 G3: 8 Season: Jan–March 2009 at baseline.	mean (95% CI) G1: −2.72 (−5.15, −0.29) G2: +33.04 (29.03, 37.06) G3: +42.90 (39.09, 46.72)			

Abbreviations: BEST-D: Biochemical Efficacy and Safety Trial of vitamin D; BMI: body mass index; CI: confidence interval; CLIA: chemiluminescence immunoassay; CPBA: competitive protein binding assay; CT: controlled trial; DO-HEALTH: Vitamin D_3 - Omega3 - Home Exercise - Healthy Ageing and Longevity Trial; EIA: enzyme immunoassay; G: group; HPLC–MS/MS: high-performance liquid chromatography tandem mass spectrometry; HR: hazard ratio; IQR: inter quartile range; IRR: incidence rate ratio; ITT: intention-to-treat; LC–MS: liquid chromatography mass spectrometry; LC–MS/MS: liquid chromatography tandem mass spectrometry; MS/MS: tandem mass spectrometry; NA; not applicable; NR: not reported; OR: odds ratio; PP: per-protocol; RECORD: Randomised Evaluation of Calcium Or vitamin D; RIA: radioimmunoassay; RCT: randomised controlled trial; RR: relative risk; SE; standard error; SEM; standard error of mean; STURDY: Study to Understand Fall Reduction and Vitamin D in You; ViDOS: Vitamin D supplementation in Older Subjects; VITAL: VITamin D and OmegA-3 TriaL; 25(OH)D: 25-hydroxyvitamin D.
(a): the values have been reported as mean ± standard deviation or median (IQR) unless otherwise indicated; IQR = 25^{th}–75^{th} percentiles or the length between the percentiles.

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C.4. Intervention studies on BMD, BMC, and indices of bone strength

Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
Children and adolescents						
Al-Shaar et al. (2013) Lebanon Latitude [assumed] 33.9° N 1 yr Private funding Based on the same study as El-Hajj Fuleihan et al. (2006) below but reported different outcomes and groups.	RCT (parallel) Inclusion criteria: Aged 10 to 17 y; healthy based on careful physical examination; absence of a history of any disorders or medications known to affect bone metabolism. Exclusion criteria: Renal disease, liver disease, chronic diarrhea, and gastric and bowel surgery; used high-dose vitamins within 6 mo of study entry; used corticosteroid therapy, anti-epileptic drugs, rifampicin, or cholestyramine. N participants randomised/ completed/analysed Females: G1: 58/55/55 G2: 62/58/58 G3: 59/55/54 Males: G1: 61/56/56	Sex (% females) All: 49.3 Age (y) Females: G1: 13.60 (11.65–15.65) G2: 12.56 (11.07–14.75) G3: 13.08 (11.13–14.72) Males: G1: 12.9 (11.8–14.8) G2: 12.6 (11.2–14.6) G3: 12.9 (11.4–14.8) Serum 25(OH)D (ng/mL) Females: G1: 11.7 (8.7–11.7) ng/ mL [29.3 (21.8–29.3) nmol/L] G2: 11.3 (8.7–15.6) ng/ mL [28.3 (21.8–39.0) nmol/L] G3: 12.6 (10.0–18.7) ng/mL [31.5 (25.0–46.8) nmol/L] Males: G1: 16.0 (12.9–19.5) ng/mL [40.0 (32.3–48.8) nmol/L]	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 1400 IU/wk [35 μg/wk = 5 μg/d] G3: vitamin D_3 14 000 IU/wk [350 μg/wk = 50 μg/d] Analysed doses: Stated that the vitamin D concentration in the 3 solutions was within 10% of that anticipated based on the label on the bottles and the dilution protocol. Background vitamin D intake: NR Background calcium intake (mg/d) Reported in the earlier publication on girls (El-Hajj Fuleihan, 2006) G1: 672 ± 323 G2: 674 ± 364 G3: 686 ± 411	BMD; DXA. Also measured in hip structural analysis: Cross sectional area, outer diameter, section modulus, and buckling ratio. **** **BMD at baseline (g/cm^2) Females** Narrow neck: G1: 0.99 ± 0.2 G2: 0.99 ± 0.2 G3: 0.97 ± 0.2 Shaft: G1: 1.38 ± 0.24 G2: 1.34 ± 0.24 G3: 1.35 ± 0.24 Intertrochanteric: G1: 0.94 ± 0.20 G2: 0.91 ± 0.15 G3: 0.89 ± 0.16	Data extracted: unclear **BMD, change over 1 yr (%), mean ± SE Females** Narrow neck: G1: 5.25 ± 0.96 G2: 7.50 ± 0.92 G3: 6.12 ± 0.98 *P* = 0.72 for difference in change between groups. [4)] Shaft: G1: 8.32 ± 0.84 G2: 9.10 ± 0.85 G3: 8.15 ± 0.84 *P* = 0.70 for difference in change between groups.[4)] Intertrochanteric: G1: 5.18 ± 0.91 G2: 8.10 ± 0.88 G3: 5.84 ± 0.92 *P* = 0.06 for difference in change between groups. [4)] [4)] adjusted for baseline height, percentage change in lean mass and height, sun	**1 for females; 2 for males**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
	G2: 59/56/55 G3: 64/60/60	G2: 15.2 (12.1–18.8) ng/mL [38.0 (30.3–47.0) nmol/L] G3: 15.5 (11.7–18.7) ng/mL [38.8 (29.3–46.8) nmol/L] BMI (kg/m^2) Females: G1: 19.9 (17.2–21.8) G2: 20.2 (17.9–23.0) G3: 19.4 (17.5–21.9) Males: G1: 20.8 (17.0–25.4) G2: 19.8 (17.3–24.5) G3: 20.3 (17.6–23.5) Ethnicity [assumed]: Arab Smoking status: NA Alcohol use: NR Health status: Healthy. Season: Recruitment occurred between Dec 2001 and June 2002.	Compliance Pill count method (%) Reported in the earlier publication on girls (El-Hajj Fuleihan, 2006) Serum 25(OH)D at 1 yr (ng/mL) G1: 16 ± 8 ng/mL [40 ± 20 nmol/L] G2: 17 ± 6 ng/mL [42.5 ± 15 nmol/L] G3: 38 ± 31 ng/mL [95 ± 77.5 nmol/L]	**Males** Narrow neck: G1: 1.01 (0.89–1.22) G2: 1.03 (0.88–1.17) G3: 0.99 (0.87–1.19) Shaft: G1: 1.38 (1.22–1.79) G2: 1.38 (1.11–1.68) G3: 1.36 (1.18–1.65) Intertrochanteric: G1: 1.05 ± 0.20 G2: 1.03 ± 0.20 G3: 1.03 ± 0.21 **** Assay of serum 25 (OH)D: CPBA (Diasorin, Incstar, Saluggia, Italy)	exposure, physical activity, calcium intake, and menarcheal status. (Table 4 presents wrong group sizes) **Males** Narrow neck: G1: 6.86 ± 0.97 G2: 5.88 ± 1.01 G3: 6.02 ± 0.93 *P* = 0.74 for difference in change between groups. [5)] Shaft: G1: 8.04 ± 0.81 G2: 8.05 ± 0.79 G3: 8.37 ± 0.75 *P* = 0.94 for difference in change between groups. [5)] Intertrochanteric: G1: 5.69 ± 0.95 G2: 5.07 ± 0.99 G3: 5.07 ± 0.92 *P* = 0.87 for difference in change between groups. [5)] [5)] adjusted for percentage change in lean mass. **Females & Males** No significant differences in %-changes in cross sectional	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
					area, outer diameter, section modulus, or buckling ratio were observed between the groups, apart from the following in girls at the narrow neck: Outer diameter, change over 1 yr (%), mean ± SE G1: 2.77 ± 0.50 G2: 0.80 ± 0.48 G3: 1.97 ± 0.51 *P* = 0.02 for difference in change between groups. [4)] Buckling ratio, change over 1 yr (%), mean ± SE G1: −1.98 ± 1.32 G2: −6.53 ± 1.26 G3: −4.16 ± 1.34 *P* = 0.049 for difference in change between groups. [4)]	
El-Hajj Fuleihan et al. (2006) Lebanon Latitude [assumed] 33.9° N 1 yr Private funding	RCT (parallel) Inclusion criteria: Children and adolescents aged 10 to 17 y; healthy based on careful physical examination and absence of a history of any disorders or medications known to affect bone metabolism.	Sex: Females Premenarcheal (%) G1: 14.5 G2: 20.7 G3: 25.5 Age (y) All G1: 13.6 ± 2.1 G2: 13.0 ± 2.1	Vitamin D_3 Doses G1: placebo G2: vitamin D_3 1400 IU/wk [35 μg/wk = 5 μg/d] G3: vitamin D_3 14 000 IU/wk [350 μg/wk = 50 μg/d] Analysed doses: Stated that the vitamin D concentration in the 3	BMD and BMC; DXA. **** **BMD at baseline:** NR **BMC at baseline (g)** **All** Total hip: G1: 24.3 ± 5.4	Data extracted: ITT Stated that the results of ITT analyses were identical with the results of PP analyses in view of the very high compliance and the fact that those who retuned for follow-up visits and BMD measurements were all	**1 for females; 2 for males**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
Based on the same study as Al-Shaar et al. (2013) above but reported different outcomes and groups	It was stated that in boys, there was no consistent positive effect of vitamin D supplementation on BMD or BMC, which was why the paper stated they reported the results mainly on girls. Exclusion criteria: Renal disease, liver disease, chronic diarrhea, and gastric and bowel surgery; used high-dose vitamins within 6 mo of study entry; used corticosteroid therapy, anti-epileptic drugs, rifampicin, or cholestyramine. N participants randomised/ completed/analysed: All (girls) G1: 58/55/55 G2: 62/58/58 G3: 59/55/55 Premenarcheal G1: NR/NR/8 G2: NR/NR/12 G3: NR/NR/14	G3: 13.1 ± 2.2 Premenarcheal G1: 10.9 ± 0.6 G2: 10.6 ± 0.6 G3: 10.8 ± 1.1 Serum 25(OH)D (ng/mL) All G1: 14 ± 7 ng/mL [35 ± 17.5 nmol/L] G2: 14 ± 9 ng/mL [35 ± 22.5 nmol/L] G3: 14 ± 8 ng/mL [35 ± 20 nmol/L] Premenarcheal G1: 13 ± 7 ng/mL [32.5 ± 17.5 nmol/L] G2: 15 ± 6 ng/mL [37.5 ± 15 nmol/L] G3: 14 ± 5 ng/mL [35 ± 12.5 nmol/L] BMI: NR Ethnicity [assumed]: Arab Smoking status: NA Alcohol use: NR Health status: Healthy.	solutions was within 10% of that anticipated based on the label on the bottles and the dilution protocol. Background vitamin D intake: NR Background calcium intake (mg/d) All G1: 672 ± 323 G2: 674 ± 364 G3: 686 ± 411 Premenarcheal G1: 805 ± 430 G2: 811 ± 383 G3: 816 ± 570 Compliance Pill count method (%) G1: 98 ± 3 G2: 98 ± 3 G3: 97 ± 3 Serum 25(OH)D at 1 yr (ng/mL) G1: 16 ± 8 ng/mL [40 ± 20 nmol/L] G2: 17 ± 6 ng/mL [42.5 ± 15 nmol/L] G3: 38 ± 31 ng/mL [95 ± 77.5 nmol/L]	G2: 22.8 ± 5.5 G3: 22.6 ± 5.9 Lumbar spine: G1: 41.1 ± 12.0 G2: 37.6 ± 10.7 G3: 39.2 ± 12.9 Femoral neck: G1: 3.3 ± 0.7 G2: 3.3 ± 0.7 G3: 3.3 ± 0.7 One-third radius: G1: 1.4 ± 0.2 G2: 1.3 ± 0.2 G3: 1.3 ± 0.3 **Premenarcheal** Total hip: G1: 17.2 ± 5.3 G2: 15.8 ± 4.2 G3: 17.0 ± 3.8 Lumbar spine: G1: 25.2 ± 6.4 G2: 25.0 ± 5.2 G3: 27.4 ± 6.3 Femoral neck: G1: 2.4 ± 0.7 G2: 2.5 ± 0.5 G3: 2.6 ± 0.5	taking the study medications. **All** **BMD, change over 1 yr (%)** Total hip: G1: 2.4 ± 4.5 G2: 4.0 ± 4.6 G3: 5.7 ± 5.8 *P* = 0.003 for difference in change between groups. Lumbar spine: G1: 4.0 ± 4.6 G2: 5.0 ± 6.3 G3: 4.3 ± 5.4 *P* = 0.61 for difference in change between groups. Femoral neck: G1: 0.7 ± 4.9 G2: 0.03 ± 4.8 G3: 0.8 ± 5.4 *P* = 0.67 for difference in % change between groups. One-third radius: NR **BMC, change over 1 yr (%)** Total hip: G1: 7.8 ± 7.7 G2: 11.2 ± 9.3 G3: 12.8 ± 10.5	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed; method of measurement Endpoint at baseline[a] Assay of serum 25(OH)D	Results[a]	RoB Tier
		Season: Recruitment occurred between Dec 2001 and June 2002.		One-third radius: G1: 1.0 ± 0.0 G2: 1.0 ± 0.2 G3: 1.0 ± 0.2 **** Assay of serum 25 (OH)D: CPBA (Diasorin, Incstar, Saluggia, Italy)	*P* = 0.02 for difference in change between groups. Lumbar spine: G1: 10.8 ± 8.5 G2: 14.5 ± 12.0 G3: 12.9 ± 10.4 *P* = 0.20 for difference in change between groups. Femoral neck: G1: 3.9 ± 7.2 G2: 4.4 ± 7.8 G3: 5.2 ± 8.0 *P* = 0.70 for difference in change between groups. One-third radius: NR **Premenarcheal** **BMD, change over 1 yr (%)** Total hip: G1: 7.4 ± 7.5 G2: 8.0 ± 4.4 G3: 12.3 ± 6.5 *P* = 0.11 for difference in change between groups. Lumbar spine: G1: 3.4 ± 7.0 G2: 3.2 ± 9.5 G3: 4.4 ± 7.7	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed; method of measurement Endpoint at baseline[a] Assay of serum 25(OH)D	Results[a]	RoB Tier
					P = 0.93 for difference in change between groups. Femoral neck: G1: 5.0 ± 3.2 G2: 2.7 ± 5.5 G3: 4.9 ± 5.2 *P* = 0.45 for difference in % change between groups. One-third radius: NR **BMC, change over 1 yr (%)** Total hip: G1: 12.3 ± 12.4 G2: 18.4 ± 9.1 G3: 23.2 ± 11.0 *P* = 0.08 for difference in change between groups. Lumbar spine: G1: 12.0 ± 9.9 G2: 18.8 ± 13.0 G3: 17.2 ± 10.2 *P* = 0.40 for difference in change between groups. Femoral neck: G1: 7.4 ± 4.5 G2: 9.3 ± 9.3 G3: 11.4 ± 7.9	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
					P = 0.50 for difference in change between groups. One-third radius: NR **Postmenarcheal** Stated there were no difference in BMD or BMC among the three treatment groups. Data not shown.	
Adults						
Burt et al. (2019) Canada Latitude [assumed] 51.05° N 3 y Private funding	RCT (parallel) Inclusion criteria: Healthy men and women aged 55–70 years; DXA lumbar spine and total hip areal BMD T score > −2.5 SD, serum 25(OH)D between 30–125 nmol/L, and normal serum calcium (2.10–2.55 mmol/L). Participants were requested to take no more than 200 IU [5 μg] per day of additional vitamin D (eg. a multivitamin supplement). Exclusion criteria: Serum25(OH)D < 30 nmol/L or > 125 nmol/L; serum calcium >2.55 mmol/L or	Sex (% females) G1: 42.9 G2: 48.5 G3: 47.5 Age (y) G1: 62.2 ± 4.2 G2: 62.6 ± 4.3 G3: 61.9 ± 4.1 Years since menopause G1: 12.6 ± 5.9 G2: 11.7 ± 7.3 G3: 12.5 ± 5.6 Serum 25(OH)D (nmol/L) G1: 76.7 ± 21.0 G2: 81.3 ± 20.1 G3: 78.4 ± 18.4 BMI (kg/m^2) G1: 27.7 ± 4.3	Vitamin D_3 Doses G1: vitamin D_3 400 IU/d [10 μg/d] G2: vitamin D_3 4000 IU/d [100 μg/d] G3: vitamin D_3 10 000 IU/d [250 μg/d] Participants not consuming the recommended dietary allowance of calcium (1200 mg/d) received calcium tablets as needed (≤600 mg/d) to approximate a total daily intake of 1200 mg. Background vitamin D intake: NR	BMD; DXA vBMD; HR-pQCT. Failure load; HR-pQCT. Also measured: Cortical porosity, trabecular number. **** **BMD at baseline (g/cm^2)** Total hip: G1: 1.02 ± 0.14 G2: 1.04 ± 0.14 G3: 1.01 ± 0.14 **vBMD at baseline (mg/cm^3)** Total, Radius: G1: 324.9 ± 61.5 G2: 335.9 ± 65.3	Data extracted: unclear **BMD at 36 mo (g/cm^2)** Total hip: G1: 1.02 ± 0.14 G2: 1.03 ± 0.15 G3: 1.00 ± 0.14 **BMD, at 36 mo (g/cm^2), mean, 95% CI** Total hip: G2 vs G1: 0.0007, −0.0064 to 0.0078 G3 vs G1: 0.0019, −0.0052 to 0.0090 P = 0.87 for group x time interaction. **vBMD at 36 mo (mg/cm^3)** Total, Radius: G1: 320.1 ± 61.1 G2: 328.6 ± 66.1	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
	<2.10 mmol/L; vitamin D supplement use >2000 IU/d [50 μg/d] for the past 6 mo; use of bone active medication within the last 2 y; disorders known to affect vitamin D metabolism such as sarcoidosis, renal failure, malabsorption disorders, kidney stone within the past 2 y, or regular use of tanning salons; DXA T score compatible with osteoporosis at the lumbar spine, total hip or femoral neck; and high 10-year risk (≥20%) for osteoporotic fracture (as defined by the World Health Organization's Canadian FRAX calculator). N participants, randomised/ completed/analysed G1: 109/100/105 G2: 100/94/97 G3: 102/93/101	G2: 28.1 ± 5.0 G3: 27.1 ± 4.1 Ethnicity (%) Non-Hispanic White: G1: 93.3 G2: 96.9 G3: 97.0 Non-Hispanic Black: G1: 1.0 G2: 1.0 G3: 0 Asian: G1: 4.7 G2: 2.1 G3: 2.0 Hispanic: G1: 1.0 G2: 0 G3: 1.0 Smokers (%) G1: 1.9 G2: 0 G3: 4.0 Alcohol use: NR Health status: NR Season: Aug 2013 to Dec 2017.	Background calcium intake: NR Compliance Pill count method – number of days of supplement administration vs total number of days (%) All: 99 on average, (range 81–100). Serum 25(OH)D at 36 mo (nmol/L) G1: 77.4 ± 17.9 G2: 132.2 ± 28.0 G3: 144.4 ± 40.4	G3: 329.7 ± 60.0 Total, Tibia: G1: 301.2 ± 58.3 G2: 314.1 ± 52.9 G3: 306.5 ± 52.6 Trabecular, Radius: G1: 163.1 ± 40.3 G2: 160.4 ± 39.9) G3: 155.9 ± 40.2 Trabecular, Tibia: G1: 176.4 ± 37.7 G2: 174.8 ± 35.2 G3: 171.9 ± 38.7 Cortical, Radius: G1: 887.6 ± 50.0 G2: 899.2 ± 51.3 G3: 904.0 ± 53.3 Cortical, Tibia: G1: 853.9 ± 61.5 G2: 868.6 ± 52.9 G3: 871.5 ± 59.0 **Failure load at baseline** Radius: G1: 2700.7 ± 1020.7 G2: 2580.1 ± 990.4 G3: 2556.5 ± 964.4 Tibia: G1: 7831.3 ± 2420.1	G3: 317.3 ± 61.7 Total, Tibia: G1: 299.1 ± 58.7 G2: 309.1 ± 54.8 G3: 301.5 ± 54.6 Trabecular, Radius: G1: 164.8 ± 42.5 G2: 161.5 ± 42.0 G3: 155.1 ± 40.1 Trabecular, Tibia: G1: 179.1 ± 40.3 G2: 178.3 ± 39.1 G3: 175.2 ± 40.8 Cortical, Radius: G1: 879.6 ± 51.0 G2: 886.3 ± 52.4 G3: 884.9 ± 54.2 Cortical, Tibia: G1: 848.1 ± 68.8 G2: 856.6 ± 62.9 G3: 853.7 ± 67.9 **vBMD, change over 36 mo (mg/cm^3), mean, 95% CI** Total, Radius G2 vs G1: −3.9, −6.5 to −1.3 G3 vs G1: −7.5, −10.1 to −5.0 $P < 0.001$ for group x time interaction.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed; method of measurement Endpoint at baseline[a] Assay of serum 25(OH)D	Results[a]	RoB Tier
				G2: 7660.6 ± 2001.2 G3: 7533.5 ± 2209.9 **** Assay of serum 25 (OH)D: CLIA (DiaSorin Liaison XL system)	Total, Tibia G2 vs G1: −1.8, −3.7 to 0.1 G3 vs G1: −4.1, −6.0 to −2.2 $P < 0.001$ for group x time interaction. Trabecular, Radius G2 vs G1: −0.95, −2.6 to 0.7 G3 vs G1: −2.71, −4.3 to −1.1 $P = 0.012$ for group x time interaction. Trabecular, Tibia G2 vs G1: 0.20, −0.1 to 1.5 G3 vs G1: −1.1, −2.4 to 0.2 $P = 0.127$ for group x time interaction. Cortical, Radius G2 vs G1: −6.3, −11.1 to −1.5 G3 vs G1: −8.6, −13.3 to −3.8 $P = 0.001$ for group x time interaction. Cortical, Tibia G2 vs G1: −4.2, −9.8 to 1.2 G3 vs G1: −9.8, −14.8 to −4.0	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
					P = 0.004 for group x time interaction. **Failure load at 36 mo** Radius: G1: 2694.8 ± 1022.7 G2: 2550.5 ± 994.3 G3: 2470.6 ± 1001.5 P = 0.12 for group x time interaction. Tibia: G1: 7785.2 ± 2443.7 G2: 7576.9 ± 2125.0 G3: 7413.0 ± 2265.1 P = 0.06 for group x time interaction.	
Grimnes et al. (2012) Norway Latitude 69° N 1 yr Private funding	RCT (parallel) Inclusion criteria: Postmenopausal women aged 50–80 years old with a T-score in total hip or lumbar spine (L2–4) ≤ −2.0. Exclusion criteria: Use of hormone replacement therapy or other therapy affecting bone remodeling during the last 12 months before enrolment; use of steroids, renal stone disease, systolic blood	Sex: Females Age (y) G1: 63.5 ± 6.8 G2: 62.9 ± 7.6 Serum 25(OH)D (nmol/L) G1: 71.2 ± 22.3 G2: 70.7 ± 23.0 BMI (kg/m^2) G1: 24.6 ± 3.2 G2: 25.0 ± 3.4 Ethnicity [assumed]: Majority Caucasian	Vitamin D_3 Doses G1: vitamin D_3 800 IU/d [20 μg/d] + calcium 1000 mg/d G2: vitamin D_3 40 000 IU/wk [5714 IU/d = 142.9 μg/d] + 800 IU/day [20 μg/d] = 6514.29 IU/d [162.9 μg/d] + calcium 1000 mg/d Background vitamin D intake (μg/d) G1: 8.1 ± 6.0 G2: 9.1 ± 6.2	BMD; DXA. **** **BMD at baseline (g/cm^2)** Total hip: G1: 0.791 ± 0.082 G2: 0.790 ± 0.073 Lumbar spine (L2-L4): G1: 0.902 ± 0.079 G2: 0.901 ± 0.072 Femoral neck G1: 0.757 ± 0.079 G2: 0.758 ± 0.066	Data extracted: ITT **BMD, change over 12 mo (%)** Total hip: G1: 0.56 ± 1.70 G2: 0.31 ± 1.59 P = 0.20 for difference in change between groups. Lumbar spine (L2-L4): G1: 0.32 ± 3.23 G2: 0.25 ± 3.19. P = 0.86 for difference in change between groups.	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[a]	Intervention[a]	Endpoint assessed; method of measurement Endpoint at baseline[a] Assay of serum 25(OH)D	Results[a]	RoB Tier
	pressure > 175 mmHg or diastolic blood pressure > 105 mmHg, serum creatinine >110 μmol/l, suspected primary hyperparathyroidism (serum calcium >2.55 mmol/l, serum calcium >2.50 mmol/l combined with plasma PTH >5.0 pmol/l, or serum calcium >2.45 mmol/l combined with plasma PTH >7.0 pmol/l), or chronic disease like ischemic heart disease, diabetes, granulomatous disease, and cancer. N participants randomised/ completed/analysed: G1: 148/140/ITT 148, PP 140 G2: 149/135/ITT 149, PP 135	Smoking status (%) Current smokers: G1: 38 G2: 37 Former smokers: G1: 39 G2: 40 Alcohol use: NR Health status (%) Asthma/COPD: G1: 10 G2: 6 Thyroid disease: G1: 9 G2: 13 Arthrosis: G1: 9 G2: 11 Other musculoskeletal disease: G1: 11 G2: 15 Hypertension: G1: 19 G2: 17 Hypercholesterolemia: G1: 11 G2: 12	Background calcium intake (mg/d) G1: 1044 ± 552 G2: 1062 ± 524 Compliance Pill count method (%) All (vitamin D): 97 All (calcium): 92. Stated no differences across groups. Serum 25(OH)D, change over 12 mo (nmol/L): G1: 18.0 ± 18.9 G2: 114.7 ± 34.6 PP population.	**** Assay of serum 25 (OH)D: LC–MS/MS (In-house, Hormone Laboratory, Haukeland University Hospital)	Femoral neck: G1: 0.17 ± 1.87 G2: 0.03 ± 2.08 *P* = 0.53 for difference in change between groups.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
Jorde et al. (2010) Norway Latitude 70° N 1 yr Mixed funding	RCT (parallel) Inclusion criteria: Males and females 21–70 y, with BMI 28.0–47.0 kg/m^2. Exclusion criteria: Diabetes or a history of coronary infarction, angina pectoris, stroke, renal stone disease, or sarcoidosis; serum calcium >2.55 mmol/L, males with serum creatinine >129 μmol/L and females with serum creatinine >104 μmol/L; use of bisphosphonates or oestrogen (for contraception or replacement); a weight loss of >10% of total body weight during the last 6 mo; use of anti-depressant drugs or weight reducing drugs; participating in an organized weight loss program; pregnancy or lactating women; women planning to become pregnant in the next 12 mo or < 50 years without adequate contraception (contraceptive pills, implantable subdermal	Sex (% females) G1: 61.0 G2: 59.8 G3: 60.0 Postmenopausal (%) G1: 55% G2: 48% G3: 44% Age (y) G1: 50.8 ± 10.7 G2: 47.7 ± 11.6 G3: 47.3 ± 11.1 Serum 25(OH)D (nmol/L) G1: 60.1 ± 22.3 G2: 58.3 ± 21.2 G3: 61.3 ± 20.7 BMI (kg/m^2) G1: 35.2 ± 3.9 G2: 33.7 ± 3.5 G3: 34.4 ± 3.9 Ethnicity [assumed]: Majority Caucasian Smokers (%) G1: 17.1 G2: 20.6 G3: 20.9 Alcohol use: NR	Vitamin D_3 Doses G1: placebo + calcium 500 mg/d G2: vitamin D_3 20 000 IU/wk [2857 IU/d = 71.4 μg/d] + calcium 500 mg/d G3: vitamin D_3 40 000 IU [5714 IU/d = 142.9 μg/d] + calcium 500 mg/d Background vitamin D intake: NR Background calcium intake: NR Compliance Pill count method – vitamin D/placebo capsules (%) G1: 96 G2: 96 G3: 95 Pill count method – calcium tablets (%) G1: 83 G2: 84 G3: 82 Serum 25(OH)D at 1 yr (nmol/L) G1: 57.9 ± 20.4	BMD; DXA. **** BMD at baseline (g/cm^2) Total hip: G1: 1.092 ± 0.130 G2: 1.067 ± 0.128 G3: 1.107 ± 0.133 Lumbar spine (L2-L4): G1: 1.251 ± 0.170 G2: 1.235 ± 0.161 G3: 1.270 ± 0.155 **** Assay of serum 25(OH)D: RIA (DiaSorin)	Data extracted: PP **BMD, change over 1 yr (g/cm^2)** Total hip G1: 0.009 ± 0.017 G2: 0.011 ± 0.014 G3: 0.008 ± 0.014 Lumbar spine (L2-L4): G1: 0.007 ± 0.042 G2: 0.008 ± 0.039 G3: 0.008 ± 0.036 *P*=NS for all comparisons vs G1 (exact values NR).	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
	contraceptive rods, contraceptive dermal patches, injectable contraceptives, vaginal contraceptive rings, or intrauterine devices, with pearl index <1.0). N participants randomised/ completed/analysed: G1: 142/105/105 G2: 132/97/97 G3: 147/110/110	Health status: Healthy overweight. Season: Recruitment occurred in Nov 2005 to October 2006.	G2: 99.7 ± 20.3 G3: 140.9 ± 34.7			
Rahme et al. (2017) Lebanon Latitude [assumed] 33.9° N 1 yr Mixed funding	RCT (parallel) Inclusion criteria: Ambulatory elderly, aged ≥65 y; overweight (BMI >25 kg/ m^2), serum 25(OH)D between 10 and 30 ng/mL [25 and 75 nmol/L] at screening. Exclusion criteria: Prediabetes if on oral hypoglycemic drugs; diabetes (fasting plasma glucose ≥126 mg/dL or glycated haemoglobin ≥6.5%); severe chronic diseases, or major organ failure (severe heart failure [stage III or IV], liver failure and cirrhosis, kidney	Sex (% females) G1: 53.6 G2: 57.3 Age (y) G1: 71.0 ± 4.7 G2: 71.2 ± 4.8 Serum 25(OH)D (ng/mL) G1: 20.0 ± 7 ng/mL [50.0 ± 17.5 nmol/L] G2: 20.9 ± 8.2 ng/mL [52.3 ± 20.5 nmol/L] BMI (kg/m^2) G1: 29.7 ± 4.6 G2: 30.6 ± 4.4 Ethnicity [assumed]: Arab	Vitamin D_3 Doses (labeled) G1: placebo + vitamin D_3 500 IU/d [12.5 μg/ d] + calcium 1000 mg/d G2: vitamin D_3 20 000 IU/wk [500 μg/wk] + vitamin D_3 500 IU/d [12.5 μg/ d] + calcium 1000 mg/ d = vitamin D_3 3357 IU/d [83.9 μg/d] + calcium 1000 mg/d Analysed vitamin D_3 doses: G1: 600 IU/d [15 μg/d] G2: 22 000 IU/wk + 600 IU/ d [15 μg/d] = 3742.86 IU/d [93.6 μg/d]	BMD; DXA. **** **BMD at baseline (g/cm^2)** Total hip: G1: 0.826 ± 0.119 G2: 0.824 ± 0.118 Lumbar spine: G1: 0.886 ± 0.154 G2: 0.890 ± 0.150 Femoral neck: G1: 0.676 ± 0.102 G2: 0.671 ± 0.101 **** Assay of serum 25 (OH)D: LC–MS (Mayo Clinic,	Data extracted: PP **BMD at 12 mo (g/cm^2)** Total hip: G1: 0.831 ± 0.121 G2: 0.828 ± 0.118 Lumbar spine: G1: 0.901 ± 0.162 G2: 0.900 ± 0.145 Femoral neck: G1: 0.682 ± 0.100 G2: 0.675 ± 0.099 **BMD, change over 12 mo (%)** Total hip: G1: 0.50 ± 2.26 G2: 0.47 ± 2.22	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
	failure [estimated glomerular filtration rate < 30 mL/min], cancer, and autoimmune diseases); conditions or being on medications known to affect bone metabolism; osteomalacia, a history of kidney stones, fragility fractures, or a 10-yr fracture risk for major osteoporotic fractures exceeding 10% based on Fracture Risk Assessment Tool Lebanon risk calculator at study entry. N participants randomised/ completed/analysed: G1: 128/112/112 G2: 129/110/110	Smoking status: NR Alcohol use: NR Health status: Overweight Season: Screening occurred between Jan 2011 and July 2013; the study ended in July 2014.	Background vitamin D intake: NR Background calcium intake (mg/d) G1: 453 ± 323 G2: 397 ± 257 Background calcium + vitamin D supplement use (%) G1: 11 ± 10 G2: 11 ± 10 <u>Compliance</u> Pill count method (%) All: >90 for both calcium and vitamin D pills, for both groups. Serum 25(OH)D at 12 mo (ng/mL) G1: 25.9 ± 6.9 ng/mL [64.8 ± 17.3 nmol/L] G2: 36.0 ± 9.7 ng/mL [90.0 ± 24.3 nmol/L]	Rochester, MN, USA)	*P* = 0.909 for difference in % change between groups. Lumbar spine: G1: 1.34 ± 3.42 G2: 1.65 ± 3.21 *P* = 0.512 for difference in % change between groups. Femoral neck: G1: 0.55 ± 3.78 G2: 0.66 ± 4.16 *P* = 0.833 for difference in % change between groups.	
Smith et al. (2018) **ViDOS** USA Latitude 41° N 1 yr	RCT (parallel) Inclusion criteria: Caucasian and African American postmenopausal women aged 57 to 90 years; vitamin D insufficiency	Sex: Females Age (y) Caucasian: 67 ± 7.3 African American: 65 ± 7.0	Vitamin D_3 Doses (labeled) G1: placebo + calcium G2: vitamin D_3 400 IU/d [10 µg/d] + calcium G3: vitamin D_3 800 IU/d [20 µg/d] + calcium	BMD; DXA **** BMD at baseline: NR ****	<u>Data extracted:</u> unclear Caucasian and African American combined: **BMD, change over 1 yr (%), mean ± SE** Femoral neck:	**1**

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
Mixed funding	defined as serum 25(OH)D ≤ 50 nmol/L. Exclusion criteria: Any significant disease or medications affecting calcium and bone metabolism: significant health problems, cancer within the last 10 y (except skin), previous hip fracture, hemiplegia, uncontrolled type 1 and type 2 diabetes, active kidney stones or history of kidney stones more than twice in their lifetime, body mass index (BMI) greater than 45 kg/m^2, serum 25(OH)D below 12.5 nmol/L, and chronic medical conditions involving liver, kidney, alcoholism or rheumatoid arthritis. Use of fluoride, bisphosphonates for more than 3 mo, PTH or derivatives within the last 6 months, calcitonin, estrogen, corticosteroid therapy of more than 10 mg/d, drugs interfering with vitamin D metabolism, such as	Serum 25(OH)D (nmol/L) Caucasian: 49.3 ± 13.3 African American: 45.0 ± 14.8 BMI: NR Ethnicity: Caucasian 84% African American 16% Smoking status: NR Alcohol use: NR Health status: NR Season: Enrolment in winter and spring over 2 years: Apr to May 2007 and Jan to May 2008. [Baseline characteristics published in the earlier publications could not be utilized here, as the results were combined regarding the ethnicities]	G4: vitamin D_3 1600 IU/d [40 μg/d] + calcium G5: vitamin D_3 2400 IU/d [60 μg/d] + calcium G6: vitamin D_3 3200 IU/d [80 μg/d] + calcium G7: vitamin D_3 4000 IU/d [100 μg/d] + calcium G8: vitamin D_3 4800 IU/d [120 μg/d] + calcium Calcium to maintain total intake between 1200 to 1400 mg/d (was based on a baseline 7-day food diary). Average calcium supplement during the study was 600 mg/d at 12 mo. Analysed vitamin D doses G2: 503 IU/d [12.6 μg/d] G3: 910 IU/d [22.8 μg/d] G4: 1532 IU/d [38.3 μg/d] G5: 2592 IU/d [64.8 μg/d] G6: 2947 IU/d [73.7 μg/d] G7: 4209 IU/d [105.2 μg/d] G8: 4937 IU/d [123.4 μg/d] Background vitamin D intake (IU/d)	Assay of serum 25(OH)D: LC–MS	G1: −0.32 ± 0.80 G2: 1.89 ± 0.85 G3: 0.23 ± 0.79 G4: 1.06 ± 0.77 G5: −0.26 ± 0.77 G6: 2.20 ± 0.82 G7: 0.72 ± 0.83 G8: 0.53 ± 0.82 *P* = 0.19 for difference in change between groups. [3)] Lumbar spine (L1-L4): G1: 0.06 ± 0.63 G2: −0.37 ± 0.67 G3: 0.24 ± 0.62 G4: 0.41 ± 0.60 G5: 0.38 ± 0.60 G6: 0.33 ± 0.67 G7: 0.25 ± 0.63 G8: 2.03 ± 0.64 *P* = 0.25 for difference in change between groups. [3)] [3)] adjusted for ethnicity.	

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Reference Study Country Duration Funding	Design	Subject characteristics at baseline[(a)]	Intervention[(a)]	Endpoint assessed; method of measurement Endpoint at baseline[(a)] Assay of serum 25(OH)D	Results[(a)]	RoB Tier
	phenytoin or phenobarbital, and high-dose thiazide therapy (>37.5 mg/d). N participants, randomised/completed/ analysed Caucasian: G1: 21/18/19 G2: 20/16/18 G3: 21/17/18 G4: 20/19/18 G5: 21/20/20 G6: 20/18/18 G7: 20/16/16 G8: 20/18/18 African American: G1: 3/3/3 G2: 2/2/2 G3: 7/4/4 G4: 5/5/5 G5: 4/4/4 G6: 3/3/3 G7: 4/4/4 G8: 3/3/3		Caucasian: 144 ± 69 IU/d [3.6 ± 1.7 μg/d] African American: 129 ± 85 IU/d [3.2 ± 2.1 μg/d] Background calcium intake (mg/d) Caucasian: 685 ± 289 African American: 524 ± 156 Compliance Pill count method - vitamin D_3 capsules (%): All: 94 on average Pill count method – calcium tablets (%) All: 91 on average Serum 25(OH)D at 1 yr: NR			

Abbreviations: BMC: bone mineral content; BMD: bone mineral density; BMI: body mass index; CI: confidence interval; CLIA: chemiluminescence immunoassay; DXA: dual-energy x-ray absorptiometry; CPBA: competitive protein binding assay; G: group; HR-pQCT: high-resolution peripheral quantitative computed tomography; ITT: intention-to-treat; LC–MS: liquid chromatography mass spectrophotometry; LC–MS/MS: liquid chromatography tandem mass spectrophotometry; NA: not applicable; NR: not reported; NS: non-significant; PP: per-protocol; RIA: radioimmunoassay; RCT: randomised controlled trial; SD: standard deviation; SE: standard error; vBMD: volumetric bone mineral density; 25(OH)D: 25-hydroxyvitamin D; y, years.

(a): the values have been reported as mean ± standard deviation or median (inter quartile range) unless otherwise indicated; interquartile range = 25^{th}–75^{th} percentiles or the length between the percentiles.

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Appendix D – Intervention studies on priority adverse health effects reported in multiple references

Several randomised controlled trials that were eligible for this assessment were reported in multiple references. In some cases, data on different endpoints were extracted from linked references, and not from the reference indicated in the forest plots or the text. Such cases are clearly indicated in text, forest plot footnotes or in the evidence tables.

Main reference and endpoints extracted	Linked references and endpoints extracted
Gallagher et al. (2012) Hypercalcaemia, Hypercalciuria	Gallagher et al. (2013); Gallagher et al. (2014a) Hypercalcaemia, Hypercalciuria
	Smith et al. (2017) Falls
	Smith et al. (2018) BMD
Burt et al. (2019) Bone fractures, Falls, BMD, bone strength	Billington et al. (2020) Hypercalcaemia, Hypercalciuria
Bischoff-Ferrari et al. (2020) Bone fractures	Bischoff-Ferrari et al. (2022) Falls
Jorde et al. (2008) Hypercalcaemia, Hypercalciuria	Sneve et al. (2008) Hypercalcaemia, Hypercalciuria
Macdonald et al. (2013) Fractures	Wood et al. (2014) Falls
El-Hajj Fuleihan et al. (2006) BMD, BMC	Al-Shaar et al. (2013) BMD
	Maalouf et al. (2008) Hypercalcaemia

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Appendix E – Studies meeting eligibility criteria for which data was not extracted

The following studies were included in the assessment but data was not extracted as described in Sections 2.1.1.3. A brief summary of these studies can be found in Section 3.5.1 for hypercalcaemia/ hypercalciuria and in Section 3.5.2.3 for BMD/BMC. A more detailed description of these studies can also be found in the technical report.

For sQ3a – hypercalcaemia and hypercalciuria.

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7) Cooper L, et al. Vitamin D supplementation and bone mineral density in early postmenopausal women. American Journal of Clinical Nutrition. 2003;77(5):1324–1329.
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18) Lagari VS, et al. Differences in vitamin D3 dosing regimens in a geriatric community-dwelling population. Endocrine Practice. 2012;18(6):847–854.

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25) Prince RL, et al. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. Archives of Internal Medicine. 2008;168(1):103–108.
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Appendix F – Dose–response meta-regression analysis and publication bias for the conversion factor of calcidiol monohydrate

This appendix describes the work done to integrate the analysis provided by the contractor and reported in the Annex F of the technical report (Lamberg-Allardt et al., 2023).

It includes two sections. The first describes the meta-regressive analysis carried out to test the possible dose–response relationship between the administered dose of vitamin D intake and the achieved serum 25(OH)D concentrations per μg/d of vitamin D (nmol/L). The second section discusses the results and challenges of the investigation for the possible presence of publication bias.

Dose response relationship

A meta-regression analysis was performed to explore the relationship between the dose of the two forms of vitamin D (calcidiol and vitamin D_3) and the achieved serum 25(OH)D concentrations per μg/d of vitamin D administered. A one-stage multivariate dose–response meta-analyses (Crippa et al., 2019) was set up.

First a linear shape was assumed including as fixed effect the dose of vitamin D (μg/d), the form of administered vitamin D (calcidiol vs. vitamin D_3) and their interactive effect. The mean baseline serum 25(OH)D concentrations (nmol/L) was used as a covariate. Studies and arms were included as nested random factors. All 26 arms from the 10 eligible RCTs were included in the model as coming from an observational setting. Therefore, the control for confounding factors normally achieved with RCTs is not necessarily attained here. The different range of doses for calcidiol and vitamin D_3 is a potential confounding only partially controlled by standardising the effect (achieved serum 25(OH)D concentrations) by unit dose. The formula describing the model is given below:

$$\boldsymbol{y_{ij}} = (\boldsymbol{\beta_0} + \boldsymbol{I_{cal}\beta_{01}}) + (\boldsymbol{\beta_1} + \boldsymbol{I_{cal}\beta_{11}}) * \boldsymbol{dose} + \boldsymbol{\beta_2} * \boldsymbol{Base_{25(OH)D}} + \boldsymbol{\varepsilon_i} + \boldsymbol{\gamma_j} \quad [1]$$

$$y_{ij} = achieved\ 25(OH)D\ by\ \mu g/d\ VitD\ in\ study\ i-th\ and\ arm\ j-th$$

$$I_{cal}\ indicator\ variable = \begin{cases} 1 & if\ arm\ with\ calcidiol \\ 0 & if\ arm\ with\ vitD3 \end{cases}$$

$$\beta_0, \beta_{01}, \beta_1, \beta_{11}, \beta_2 = regression\ parameters\ for\ the\ fixed\ effects$$

$$\varepsilon_i, \gamma_j = random\ effects\ due\ to\ study\ and\ arm\ respectively$$

The goodness of fit of the model was assessed considering: 1. the Akaike Information Criteria – AIC (Akaike, 1974); 2. significance of the parameters; 3. the explained heterogeneity. The results of the model 1 estimates are provided in Table F.1.

Table F.1: Meta-regressive linear dose–response model 1

Parameter	Estimate	Se	CI lower bound	CI Upper bound
Intercept vitamin D_3	8.04	21.74	−37.16	53.24
Dose vitamin D_3	−0.07	0.23	−0.55	0.42
Intercept calcidiol (expressed as differential intercept from vitamin D_3)	6.30	11.23	−17.05	29.64
Dose calcidiol (expressed as differential parameter from vitamin D_3)	−0.15	0.67	−1.54	1.24
Baseline serum 25(OH)D	−0.07	0.51	−1.14	1.00

The model does not support any effect of the dose (for both vitamin D_3 and calcidiol), the type of vitamin D administered, or baseline serum 25(OH)D concentrations on achieved serum 25(OH)D concentrations per μg/day at the end of the study. This might be due to the large sampling uncertainty rather than to the residual heterogeneity, which is not significantly different from zero (Q = 0.04, p = 1.0).

Dropping baseline serum 25(OH)D (model 2 – Table F.2) and the interactive effect between the dose and the vitamin D type (model 3 – Table F.3) from the list of predictors of the model did not lead to evidence of an effect of the dose or the vitamin D type on the dependent variable.

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Table F.2: Meta-regressive linear dose–response model 2

Parameter	Estimate	Se	CI lower bound	CI Upper bound
Intercept vitamin D_3	5.29	5.34	−5.79	16.36
Dose vitamin D_3	−0.07	0.23	−0.55	0.42
Intercept calcidiol (expressed as differential intercept from vitamin D_3)	5.99	10.97	−16.77	28.75
Dose calcidiol (expressed as differential parameter from vitamin D_3)	−0.12	0.63	−1.43	1.19

Table F.3: Meta-regressive linear dose–response model 3

Parameter	Estimate	Se	CI lower bound	CI Upper bound
Intercept vitamin D_3	5.64	5.01	−4.73	16.01
Intercept calcidiol (expressed as differential intercept from vitamin D_3)	4.09	4.75	−5.73	13.92
Dose vitamin D_3	−0.08	0.22	−0.53	0.36

No evidence for a dose–response relationship was observed either when testing for a non-linear shape (quadratic) – model 4 (Table F.4). The goodness of fit of the four models is reported in Table F.5.

Table F.4: Meta-regressive non-linear dose–response model 4

Parameter	Estimate	Se	CI lower bound	CI Upper bound
Intercept vitamin D_3	−3.84	75.26	−160.83	153.16
Intercept calcidiol (expressed as differential intercept from vitamin D_3)	15.78	78.03	−146.99	178.56
Dose vitamin D_3	0.54	4.99	−9.86	10.94
Dose calcidiol (expressed as differential parameter from vitamin D_3)	−0.84	5.84	−13.01	11.34
Dose vitamin D_3 quadratic	−0.007	0.06	−0.14	0.12
Dose calcidiol quadratic (expressed as differential parameter from vitamin D_3)	0.011	0.11	−0.23	0.25

Table F.5: Goodness of fit of the 4 models testing the dose–response relationship

Model	logLik	Deviance	AIC	BIC	AICc
Model 1	−79.53	159.06	173.06	180.38	181.68
Model 2 (no baseline)	−83.25	166.49	178.49	185.04	184.09
Model 3 (no interactive effect dose*vitamin D type)	−87.01	174.01	184.01	189.70	187.54
Model 4 (quadratic shape)	−75.37	150.73	166.73	174.70	179.82

The analysis done by the contractor to simulate the predicted ratio of means (RoM) based on model 1 and described in Annex F of the technical report is not discussed, owing to the lack of statistical significance of all the parameters of the model.

Publication bias

Funnel plots are traditionally used to explore whether publication bias could affect the body of evidence (Sterne et al., 2011) displaying the effect estimate standard error (SE) towards the study's effect (RoM between calcidiol and vitamin D_3 arms in this case). An asymmetric funnel indicates a relationship between the effect estimate and study precision: this suggests the possibility of either publication bias or a systematic difference between studies with higher and lower precision (highly correlated to study size). Studies falling out of the funnel might be indicative of publication bias. To explore quantitatively the presence of publication bias, the Egger test is generally performed.

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However, it has been highlighted that the use of funnel plot and Egger test can be misleading when a continuous outcome is considered (Higgins et al., 2022). In this case, a *baseline risk interaction* can be present, i.e. a dependence of the measure of effect (i.e. RoM) on the comparator's outcome level (i.e. the achieved serum 25(OH)D for vitamin D_3 arms) possibly leading to an asymmetry of the funnel plot and significance of the Egger test, even in the absence of publication bias. Although some methods have been proposed to explore the possible presence of publication bias when also the baseline risk interaction is present, those methods have shown to lack power to detect publication bias in most cases particularly with a limited number of studies.

Owing to these considerations, the possible presence of a baseline risk interaction was explored. Figure F.1 displays the scatter plot of the measure of effect (RoM) versus the level of the outcome in the comparator (achieved 25(OH)D when vitamin D_3 is administered). Each point represents a comparison between calcidiol and vitamin D_3. Arms from the same study share the same colour. The shape identifies the dose of vitamin D_3 administered; the size of the dots is indicative of the sample size of the comparator arm.

The plot shows a strong dependency of the RoM on the comparator level (the so-called baseline risk interaction) mainly driven by arms with high levels of vitamin D_3 as comparator (top left) and the arm with a dose of 25 μg/d (bottom right). They correspond to the dots in the extreme right and the top left in the funnel plot (Figure F.2). The correlation between the RoM and outcome level in the comparator is −0.89.

Heterogeneity in the results seems to depend more on the dose used for the comparator (vitamin D_3) than on the size of the study. This makes difficult to assess the presence of publication bias. Inspection of the funnel plot and analysis of the results of the Egger test are not recommended in these situations.

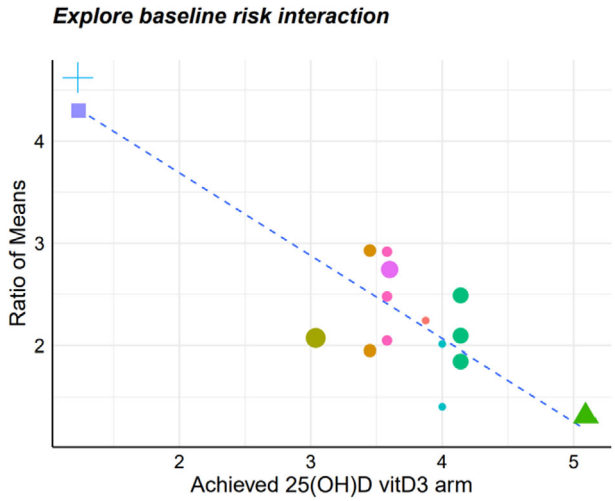

Figure F.1: Scatter plot of the Ratio of Means (RoM) versus the achieved 25(OH)D per unit dose (nmol/L) of the comparator (vitamin D_3)

Legend to Figure F.1: the colours represent studies; the shape indicates the dose of the control group (dot = 20 μg/d, triangle = 25 μg/d, square = 60 μg/d, cross = 62.5 μg/d); the size of the points is proportional to the sample size of the control group (vitamin D_3).

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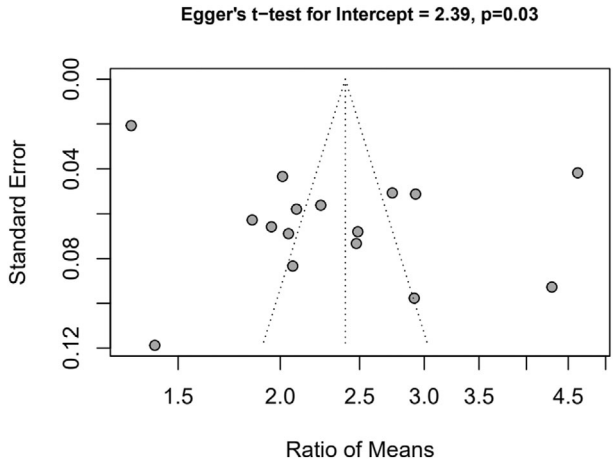

Figure F.2: Funnel plot of the 16 arms from the 10 eligible studies

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Appendix G – National provisions on the mandatory and voluntary addition of vitamin D to foods and national nutritional guidelines/recommendations for supplementing the diet with vitamin D

Table G.1: National provisions on the mandatory addition of vitamin D to food

Country	Food	Amount
Sweden	Drinking milk with a fat content not exceeding 3.0% by weight of fat (m/m). The vitamin content requirement shall also apply to vegetable and lactose-free products with a fat content not exceeding 3.0% (m/m) which are intended for use as An alternative to drinking milk. Flavored products or products with added sugars or sweeteners are not covered by the provision. Certain exceptions apply.	0.95–1.10 μg/100 g
Sweden	Fermented milk products with a fat content not exceeding 3.0% by weight of fat (m/m). The requirement shall not apply to cheese. The vitamin content requirement shall also apply to plant products with a fat content not exceeding 3.0% (m/m) fat and intended for use as an alternative to fermented milk products. Certain exceptions apply.	0.75–1.10 μg/ 100 g
Sweden	Margarine and fat blends. Also applies to liquid products and products with other fat content which otherwise correspond to margarine or fat blends. Certain exceptions apply.	19.5–21.0 μg/100 g
Belgium	Margarine, low-fat margarine and fats for baking	2.5–3 International Units (IU)[1] per gram
Finland	Skimmed homogenized milk and organic milk	1 μg/100 ml
Poland	Margarine with normal and reduced contents of fat, mixture of butter and oil	≤ 7.5 μg/100 g

Table G.2: National covenants

Country	Food	Amount
The Netherlands	Margarines and other spreadable fats, and baking fat products (excl. oils and 100% fat)	0.056–0.075 μg/g

Table G.3: National voluntary vitamin D fortification policies

Country	Food	Amount
Germany	Margarine and margarine-like spreads	≤ 25 μg/kg
Finland	Milk and fermented milk and yogurts as well as vegetable based drinks used as substitutes for these	1 μg/100 ml
Finland	Spreadable fats	20 μg/100 g
Norway	Margarine, cooking oils and other fats for eating Butter Milk (to consumers)	10 μg/100 g 10 μg/ 100 g 0,4 μg/100 g
The Netherlands	Fats, liquid products with the same purpose, baking and frying products	Max. 0.075 μg/g

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Country	Food	Amount
The Netherlands	Yellow fat spreads intended for persons of 60 years and older	0.20–0.25 μg/g
The Netherlands	Foodstuffs and beverages	Max. 4.5 μg/100 kcal

Table G.4: National nutritional guidelines/recommendations for supplementing the diet with vitamin D

Country	Food	Population group	Amount
Belgium	Food supplements	Patients with osteoporosis	1 to 1.2 g/day
Belgium	Food supplements	From birth and throughout childhood	Up to 10 μg/day
Belgium	Food supplements or fortified foods	Take more vitamin D in the winter period	Not specified
Belgium	Food supplements or fortified foods	vegans	Not specified
Germany	Food supplements	Groups at risk of insufficiency, including people with dark skin, veiled people, frail elderly people	Not specified
Denmark	Food supplements	Infants from 2 weeks of age and young children	10 μg/day
Denmark	Food supplements	Children from 4 years of age	5–10 μg/day (only October–April)
Denmark	Food supplements	Adults until 70 years of age	5–10 μg/day (only October–April)
Denmark	Food supplements	Adults above 70 years of age	20 μg/day
Denmark	Food supplements	Adults in nursing homes	20 μg/day
Denmark	Food supplements	People with increased risk of osteoporosis	20 μg/day
Denmark	Food supplements	Pregnant women	10 μg/day (throughout pregnancy)
Denmark	Food supplements	Children and adults with dark skin, or who avoid sunlight, or who wear covering attire	10 μg/day
Denmark	Food supplements	Adults who follow a vegan diet	10 μg/day (only October–April)
Denmark	Food supplements	Children from 2 years of age who follow a vegan diet	10 μg/day
Estonia	Food supplements	Age group: 2 weeks-24 months	10 μg/day
Estonia	Food supplements or fortified foods	Age group: 2–60 years	up to 10 μg/day
Estonia	Food supplements or fortified foods	Age group: >60 years	up to 20 μg/day
Finland	Food supplements	Age group: 2 weeks to 12 months	10 μg/day (2–10 μg depending on the individual feeding, breast milk or amount of infant formula used)
Finland	Food supplements	Age group: 1 - < 2 years	10 μg/day

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Country	Food	Population group	Amount
Finland	Food supplements	Age group: 2–17 years	7.5 μg/day
Finland	Food supplements	Age group: 18–60 years	10 μg/day (If dairy fortified products, fat spreads and/or fish are not used daily in the darkest time of the year (October–March).
Finland	Food supplements	Age group: 61–74 years	10 μg/day (If dairy fortified products, Fat spreads and/or fish are not used daily in the darkest time of the year (October–March).
Finland	Food supplements	Individuals, who stay outside very little, are covertly dressed or dark-skinned	20 μg/day year-round
Finland	Food supplements	>75 years	20 μg/day (A smaller dose may be enough for those who regularly use large quantities of fortified milk products fat spreads and/or fish)
Finland	Food supplements	Pregnant and lactating women	10 μg/day
Norway	Food supplements	Elderly and those with little or no access to sun exposure	10 μg/day for adults, 20 μg/day for elderly
Poland	Food supplements	Children 1–10 years	15–25 μg/day (600–1000 IU/day) If insolation guidelines are not fulfilled
Poland	Food supplements	Adolescents 11–18 years and adults 19–65 years	20–50 μg/day (800–2000 IU/day) If insolation guidelines are not fulfilled
Poland	Food supplements	Adults >65–75 years and people with a dark complexion	20–50 μg/day (800–2000 IU/day)
Poland	Food supplements	Adults >75 years	50–100 μg/day (2000–4000 IU/day)
Poland	Food supplements	Pregnant and breastfeeding women without factors suggesting vitamin D deficiency	37.5–50 μg/day (1500–2000 IU/day)
Norway	Food supplements	Vegans	Not specified
Sweden	Food supplements	Children up to two years of age	10 μg/day
Sweden	Food supplements	Children and adults who do not eat fish and vitamin D-fortified foods,	10 μg/day
Sweden	Food supplements	Children and adults with limited sun exposure	10–20 μg/day
Sweden	Food supplements	Older adults >75 years	20 μg/day
The Netherlands	Food supplements	Women 4–49 years with dark complexion (tinted)	10 μg/day
The Netherlands	Food supplements	Women 4–49 years who do not get much sun during the day or cover their skin	10 μg/day
The Netherlands	Food supplements	≥ 70 years	20 μg/day
The Netherlands	Food supplements	Pregnant women	10 μg/day
The Netherlands	Food supplements	Men 4–69 years with dark complexion (tinted)	10 μg/day

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Country	Food	Population group	Amount
The Netherlands	Food supplements	Men 4–69 years who do not get much sun during the day or cover their skin	10 μg/day
Ireland	Food supplements	Infants from birth to 12 months if they are breastfed or taking less than 300 ml or 10 fluid oz (ounces) of infant formula a day	5 μg/day
Ireland	Food supplements	Children 1–4 years during winter months (October–March)	5 μg/day
Ireland	Food supplements	Older adults ≥65 years	15 μg/day

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List of Annexes

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Annex A – Protocol for the Scientific opinion on the Tolerable Upper Intake Level for vitamin D, including the derivation of a conversion factor for calcidiol monohydrate

Annex B – EFSA's intake assessment for vitamin D

Annex C – Methodological considerations in the calculation of intake estimates for vitamin D in European countries

Annex D – Intake data from Competent Authorities in European countries

Annex E – Outcome of the public consultation on the draft scientific opinion on the tolerable upper intake level for vitamin D, including the derivation of a conversion factor for calcidiol monohydrate

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