

ADOPTED: 5 July 2023

doi: 10.2903/j.efsa.2023.8145

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

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA),  
Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan de Henauw,  
Karen-Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf,  
Harry J McArdle, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti,  
Susan Lanham-New, Giovanni Passeri, Ionut Craciun, Lucia Fabiani, Rita Ferreira De Sousa,  
Laura Martino, Silvia Valtueña Martínez and Androniki Naska

### 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<sub>3</sub> for labelling purposes. Vitamin D refers to ergocalciferol (vitamin D<sub>2</sub>), cholecalciferol (vitamin D<sub>3</sub>), and calcidiol monohydrate. Systematic reviews of the literature were conducted to assess the relative bioavailability of calcidiol monohydrate versus vitamin D<sub>3</sub> 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.

© 2023 European Food Safety Authority. *EFSA Journal* published by Wiley-VCH GmbH on behalf of European Food Safety Authority.

**Keywords:** Ergocalciferol, cholecalciferol, calcidiol monohydrate, conversion factor, tolerable upper intake level

**Requestor:** European Commission

**Question numbers:** EFSA-Q-2021-00367, EFSA-Q-2022-00215

**Correspondence:** [nif@efsa.europa.eu](mailto:nif@efsa.europa.eu)

**Panel members:** Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact [interestmanagement@efsa.europa.eu](mailto:interestmanagement@efsa.europa.eu).

**Acknowledgments:** The Panel wishes to thank for their contribution to this output: the WG on Upper Levels: Peter Aggett, Brandy Beverly, Torsten Bohn, Julia Bornhorst, Marta Crous Bou, Francesco Cubadda, Aymeric Dopter, Susan Fairweather-Tait, Rex FitzGerald, Susan Lanham New, Georg Lietz, Harry J McArdle, Anne Molloy, Androniki Naska, Giovanni Passeri, Kristina Pentieva, Marco Vinceti and Misha Vrolijk; the hearing experts Roger Bouillon and Paul Lips; and EFSA staff members: Pedro Das Neves Ferreira, Agnès de Sesmaisons Lecarré, Thibault Fiolet, Irene Muñoz Guajardo, Franco Neri, Roanne Marie Saad and Angeliki Sofroniou. The Panel also wishes to thank Carmen Peláez for her contribution as member of the NDA Panel until June 2023. The Panel wishes to acknowledge Folasade Adebayo, Jacob J Christiansen, Christel Lamberg-Allardt, Thomas Olsen and Henna Peltonen for the preparatory work as part of a procurement procedure. The Panel also wishes to acknowledge the contribution of all national institutions in European countries that provided consumption data for this scientific output and the authors of published papers on vitamin D who provided additional information upon request.

**Suggested citation:** EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Bohn, T., Castenmiller, J., de Henauw, S., Hirsch-Ernst, K.-I., Knutsen, H. K., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Lanham-New, S., Passeri, G., Craciun, I., Fabiani, L., ... Naska, A. (2023). Scientific opinion on the tolerable upper intake level for vitamin D, including the derivation of a conversion factor for calcidiol monohydrate. *EFSA Journal*, 21(8), 1–219 pp. <http://doi.org/10.2903/j.efsa.2023.8145>

**ISSN:** 1831-4732

© 2023 European Food Safety Authority. *EFSA Journal* published by Wiley-VCH GmbH on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

EFSA may include images or other content for which it does not hold copyright. In such cases, EFSA indicates the copyright holder and users should seek permission to reproduce the content from the original source.



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



## Table of contents

|   |    |
|---|----|
| Abstract.....   | 1  |
| 1. Introduction.....  | 5  |
| 1.1. Background as provided by the European Commission.....   | 5  |
| 1.1.1. Tolerable upper intake level for vitamin D.....  | 5  |
| 1.1.2. Conversion factor for calcidiol monohydrate.....   | 5  |
| 1.2. Terms of reference as provided by the European Commission.....   | 6  |
| 1.2.1. Tolerable upper intake level for vitamin D.....  | 6  |
| 1.2.2. Conversion factor for calcidiol monohydrate.....   | 6  |
| 1.3. Overview of previous assessments of the UL for vitamin D.....  | 7  |
| 1.4. Other assessments of vitamin D by EFSA.....  | 7  |
| 1.5. Interpretation of the terms of reference and context of the assessment.....                            | 9  |
| 2. Data and methodologies.....  | 9  |
| 2.1. Hazard identification and characterisation.....  | 11 |
| 2.1.1. Data.....  | 11 |
| 2.1.1.1. Literature searches.....   | 11 |
| 2.1.1.2. Study selection.....   | 11 |
| 2.1.1.3. Data extraction.....   | 12 |
| 2.1.1.4. Requests for additional information.....   | 13 |
| 2.1.2. Methodologies.....   | 13 |
| 2.1.2.1. Evidence appraisal (sQ1c, sQ3, sQ4).....   | 13 |
| 2.1.2.2. Evidence synthesis (sQ1c, sQ3, sQ4, sQ5).....  | 14 |
| 2.1.2.3. Evidence integration and uncertainty analysis (sQ1c, sQ3, sQ4, sQ5).....                           | 14 |
| 2.2. Dietary intake assessment.....   | 14 |
| 2.2.1. Data.....  | 14 |
| 2.2.2. Methodologies.....   | 15 |
| 3. Assessment.....  | 16 |
| 3.1. Chemistry of vitamin D.....  | 16 |
| 3.2. Physiology and metabolism.....   | 18 |
| 3.2.1. Cutaneous synthesis.....   | 18 |
| 3.2.2. Intestinal absorption.....   | 18 |
| 3.2.3. Transport in blood.....  | 18 |
| 3.2.4. Distribution to tissues.....   | 19 |
| 3.2.5. Storage.....   | 19 |
| 3.2.6. Metabolism.....  | 19 |
| 3.2.7. Elimination.....   | 20 |
| 3.3. Biomarkers of intake and status.....   | 20 |
| 3.3.1. Serum concentration of 25(OH)D as marker of vitamin D intake and status.....                         | 20 |
| 3.3.1.1. Methods of measurement of 25(OH)D in plasma/serum.....   | 20 |
| 3.3.1.2. Dose–response between vitamin D intake and serum 25(OH)D.....                                      | 21 |
| 3.3.1.3. Factors affecting vitamin D status.....  | 22 |
| 3.3.2. Serum parathyroid hormone concentration.....   | 23 |
| 3.3.3. Other biomarkers.....  | 24 |
| 3.3.4. Conversion factor for calcidiol monohydrate into vitamin D <sub>3</sub> .....                        | 24 |
| 3.3.4.1. Body of evidence.....  | 24 |
| 3.3.4.2. Effect of calcidiol monohydrate versus vitamin D <sub>3</sub> on serum 25(OH)D concentrations..... | 25 |
| 3.3.4.3. Effect of calcidiol monohydrate versus vitamin D <sub>3</sub> on serum PTH concentrations.....     | 31 |
| 3.3.4.4. Derivation of a conversion factor for calcidiol monohydrate.....                                   | 33 |
| 3.3.4.5. Uncertainty analysis.....  | 33 |
| 3.4. Intake assessment.....   | 34 |
| 3.4.1. Dietary sources of vitamin D.....  | 34 |
| 3.4.2. EFSA's intake assessment on background intake.....   | 35 |
| 3.4.2.1. Sources of uncertainty.....  | 39 |
| 3.4.3. Information on fortified foods and food supplements.....   | 39 |
| 3.4.3.1. Intake from fortified foods.....   | 39 |
| 3.4.3.2. Intake from food supplements.....  | 40 |
| 3.4.4. Overall conclusions on intake data.....  | 43 |
| 3.5. Hazard identification.....   | 43 |
| 3.5.1. Persistent hypercalcaemia and hypercalciuria.....  | 43 |
| 3.5.1.1. Children and adolescents.....  | 45 |
| 3.5.1.2. Pregnant and lactating women.....  | 46 |
| 3.5.1.3. Adults.....  | 47 |

|          |   |     |
|----------|---|-----|
| 3.5.1.4. | Mechanisms of toxicity .....  | 54  |
| 3.5.1.5. | Evidence integration and uncertainty analysis.....  | 55  |
| 3.5.1.6. | Conclusions on persistent hypercalcaemia and hypercalciuria .....   | 55  |
| 3.5.2.   | Musculoskeletal health .....  | 56  |
| 3.5.2.1. | Bone fractures (sQ4a) .....   | 56  |
| 3.5.2.2. | Falls (sQ4b).....   | 58  |
| 3.5.2.3. | BMD/BMC and indices of bone strength (sQ4c) .....   | 60  |
| 3.5.2.4. | Mechanisms of toxicity .....  | 63  |
| 3.5.2.5. | Evidence integration and uncertainty analysis.....  | 64  |
| 3.5.2.6. | Conclusions on musculoskeletal health.....  | 65  |
| 3.5.3.   | Other adverse health effects .....  | 65  |
| 3.5.3.1. | Kidney stones .....   | 65  |
| 3.5.3.2. | Cardiovascular disease .....  | 66  |
| 3.5.3.3. | Cancer .....  | 67  |
| 3.5.3.4. | All-cause mortality .....   | 68  |
| 3.6.     | Hazard characterisation .....   | 69  |
| 3.6.1.   | Selection of the critical effect .....  | 69  |
| 3.6.2.   | Derivation of the UL .....  | 70  |
| 3.6.2.1. | Adults .....  | 70  |
| 3.6.2.2. | Pregnant and lactating women .....  | 71  |
| 3.6.2.3. | Children and adolescents.....   | 71  |
| 3.7.     | Risk characterisation .....   | 71  |
| 4.       | Conclusions.....  | 72  |
| 5.       | Recommendations for research .....  | 72  |
|          | References.....   | 73  |
|          | Abbreviations .....   | 88  |
|          | Glossary .....  | 89  |
|          | Appendix A – Flow charts for the selection of studies.....  | 91  |
|          | Appendix B – Outcome of the appraisal for the risk of bias of included studies .....  | 94  |
|          | Appendix C – Evidence tables for the studies included.....  | 99  |
|          | Appendix D – Intervention studies on priority adverse health effects reported in multiple references .....  | 206 |
|          | Appendix E – Studies meeting eligibility criteria for which data was not extracted .....  | 207 |
|          | Appendix F – Dose–response meta-regression analysis and publication bias for the conversion factor of calcidiol monohydrate.....  | 211 |
|          | 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..... | 215 |
|          | List of Annexes .....   | 219 |



## 1. Introduction

Directive 2002/46/EC<sup>1</sup> on food supplements and Regulation (EC) No 1925/2006<sup>2</sup> 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.<sup>3</sup>

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<sub>3</sub>, and to derive a conversion factor that allows to convert absolute amounts of this nutrient form in µg into µg of native vitamin D<sub>3</sub>. 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,<sup>4</sup> folic acid/folate,<sup>4</sup> vitamin D,<sup>4</sup> vitamin E,<sup>4</sup> vitamin B6, iron,<sup>4</sup> manganese<sup>4</sup> and β-carotene<sup>4</sup> to take into account recent scientific developments and evidence.

In this context, EFSA should first review the guidelines of the SCF<sup>4</sup> 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<sup>5</sup>, including its bioavailability as a metabolite of vitamin D<sub>3</sub> when added for nutritional purposes to food supplements (EFSA NDA Panel, 2021a).

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> EFSA Mandate No M-2021-00058 of 7 June 2021.

<sup>4</sup> 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.

<sup>5</sup> 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.

In its Scientific Opinion, EFSA concluded that calcidiol monohydrate is safe under the proposed conditions of use and use levels for individuals  $\geq 11$  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<sub>3</sub> 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  $\mu\text{g}/\text{day}$ . 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<sub>3</sub> might cause difficulties for the national competent authorities in enforcing compliance with the abovementioned provision.

In addition, both Regulation (EU) No 1169/2011<sup>6</sup> 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.
  - $\beta$ -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<sub>3</sub>, as well as to derive a conversion factor that allows to convert absolute amounts in  $\mu\text{g}$  of this nutrient form into  $\mu\text{g}$  of vitamin D<sub>3</sub>.

<sup>6</sup> 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.

### 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) |
|---------------------------------|------------------------------|--------------------|-------------------|-------------|
| <b>Infants</b>                  |                              |                    |                   |             |
| 0–6 months                      | 25                           | 25                 | 25                | 25          |
| 7–11 months <sup>(1)</sup>      | 35                           | 38                 | 25                | 25          |
| <b>Children and adolescents</b> |                              |                    |                   |             |
| 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 <sup>(2)</sup> | 80 <sup>(2)</sup> |             |
| 15–17 years                     | 100 <sup>(2)</sup>           |                    |                   | 100         |
| <b>Adults</b>                   |                              |                    |                   |             |
| ≥ 18 years                      | 100 <sup>(2)</sup>           |                    |                   | 100         |
| ≥ 19 years                      |                              | 100 <sup>(2)</sup> | 80 <sup>(2)</sup> |             |

(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.

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<sub>3</sub>), 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<sub>3</sub>), also called calcidiol, calcidiol monohydrate, calcifediol, or 25-hydroxy vitamin D<sub>3</sub>, was assessed by the NDA Panel as novel food pursuant to Regulation (EU) 2015/2283<sup>7</sup> (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<sub>2</sub> to vitamin D<sub>2</sub>. 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<sub>3</sub> (10 µg/day in adults, 5 µg/day in children), which was estimated using a biological activity factor relative to vitamin D<sub>3</sub> 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<sub>3</sub> in animal feed would not present a risk for the consumer.

The FEEDAP Panel assessed the safety of vitamin D<sub>3</sub> 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).

<sup>7</sup> 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.

## 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<sub>3</sub>), ergocalciferol (vitamin D<sub>2</sub>) 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<sup>8</sup> 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<sub>3</sub>) compared to native vitamin D<sub>3</sub> 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.

<sup>8</sup> 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).



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

| No.        | Sub-question   | Methods  |
|------------|--|--|
| <b>sQ1</b> | <b>ADME of the different forms of vitamin D</b>  |  |
|            | <b>sQ1a.</b> What is the ADME of the different forms of vitamin D in humans?   | Narrative review                                     |
|            | <b>sQ1b.</b> Are there differences related to age, vitamin D status, or other individual factors, e.g. genetic polymorphisms?  | Narrative review                                     |
|            | <b>sQ1c.</b> What is the relative bioavailability of calcidiol monohydrate (25(OH)D <sub>3</sub> ) compared to native vitamin D <sub>3</sub> ? Could a conversion factor be derived for use across population groups and vitamin D <sub>3</sub> doses? | Systematic review                                    |
| <b>sQ2</b> | <b>Biomarkers of exposure for vitamin D</b>  |  |
|            | <b>sQ2a.</b> What is the dose–response relationship between vitamin D intake and serum 25(OH)D concentrations?   | Narrative review                                     |
|            | <b>sQ2b.</b> Are there differences related to age, sex, or other individual factors (e.g. genetic polymorphisms?)  | Narrative review                                     |
| <b>sQ3</b> | <b>Persistent hypercalcaemia/hypercalciuria</b>  |  |
|            | <b>sQ3a.</b> 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                                    |
|            | <b>sQ3b.</b> 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                                     |
| <b>sQ4</b> | <b>Musculoskeletal health</b>  |  |
|            | <b>sQ4a.</b> 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                                    |
|            | <b>sQ4b.</b> 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                                    |
|            | <b>sQ4c.</b> 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                                    |
|            | <b>sQ4d.</b> 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                                     |
| <b>sQ5</b> | What <b>other adverse health effects</b> have been reported to be associated with ‘high’ intake of vitamin D?  | Narrative review                                     |
| <b>sQ6</b> | <b>Vitamin D intake</b>  |  |
|            | <b>sQ6a.</b> 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 |
|            | <b>sQ6b.</b> 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?   | Food composition and food consumption data 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).

## 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<sup>®</sup> (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<sup>®</sup> 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<sub>3</sub> 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).



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  $\geq 15$   $\mu\text{g}/\text{day}$  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<sup>®</sup> by two extractors and were jointly discussed, compared and harmonised at several time points. Evidence tables were prepared in Microsoft Word<sup>®</sup> and are provided in Appendix C.

For sQ3a, data extraction was restricted to studies with at least one arm with a vitamin D dose  $\geq 100$   $\mu\text{g}/\text{day}$  in adults and  $\geq 50$   $\mu\text{g}/\text{day}$  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  $< 100$   $\mu\text{g}/\text{day}$  for adults

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

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

|               |  |                 |
|---------------|--|-----------------|
| <b>Tier 3</b> | Study must be rated as “definitely high” -- or “probably high” -/NR risk of bias for most (at least two) <i>key criteria</i> AND have most other applicable criteria rated as “definitely high” -- or “probably high” -/NR risk of bias. | <b>High RoB</b> |
|---------------|--|-----------------|

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<sup>2</sup> 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.

### *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)<sup>9</sup> 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

<sup>9</sup> 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>

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<sub>3</sub>), ergocalciferol (vitamin D<sub>2</sub>) 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.<sup>10</sup>

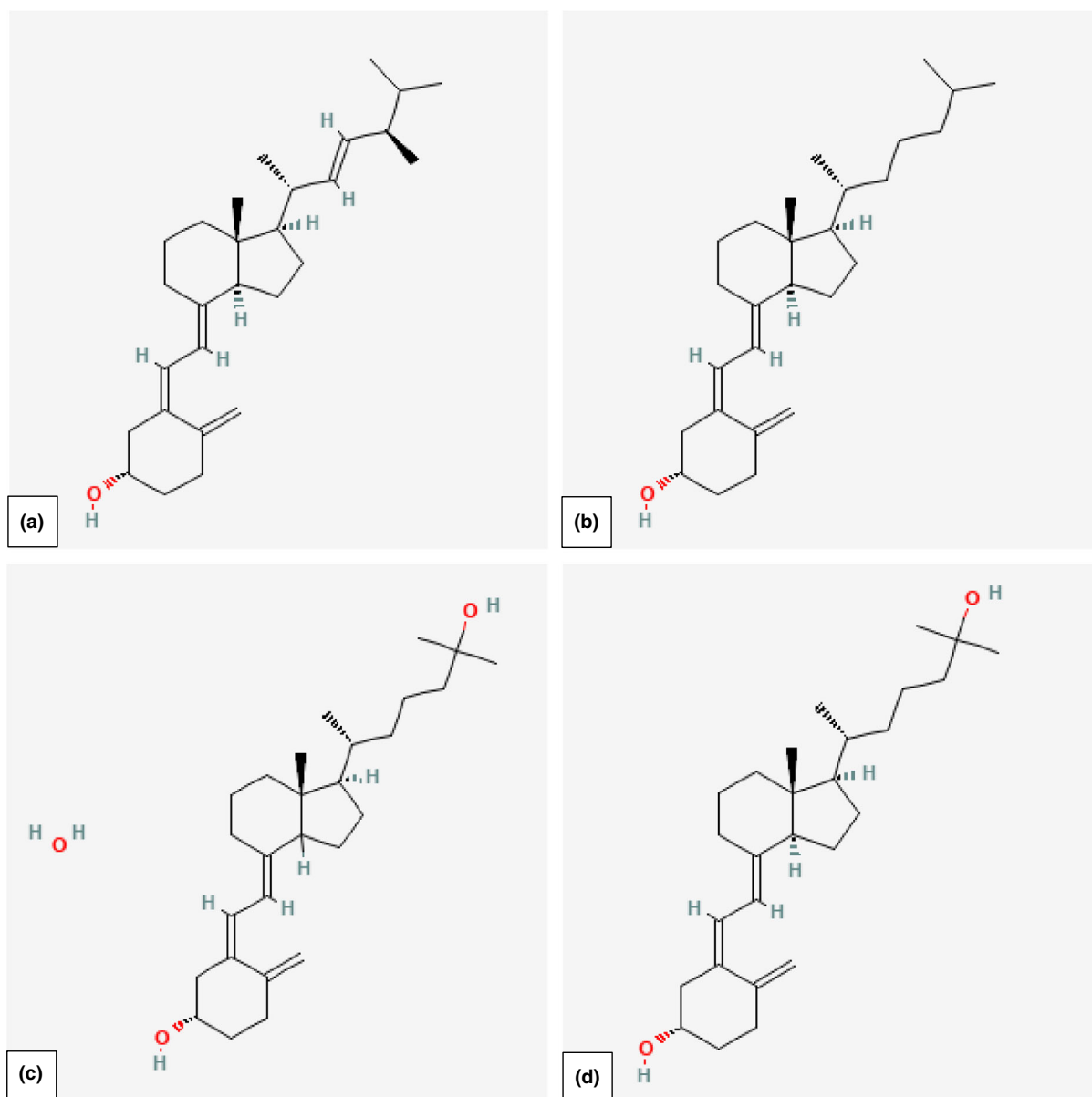
In the body, vitamins D<sub>2</sub> and D<sub>3</sub> are converted to the main circulating forms, 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, called calcidiols, and these to the active metabolites 1,25(OH)<sub>2</sub>D<sub>2</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>, called calcitriols. In this opinion, the term 25(OH)D refers to both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D refers to both 1,25(OH)<sub>2</sub>D<sub>2</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> unless the specific form is indicated.

#### 3.1. Chemistry of vitamin D

Vitamin D is the generic term for ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>), 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<sub>2</sub> (C<sub>28</sub>H<sub>44</sub>O) differs from vitamin D<sub>3</sub> (C<sub>27</sub>H<sub>44</sub>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<sub>27</sub>H<sub>46</sub>O<sub>3</sub>) is obtained by chemical synthesis (EFSA NDA Panel, 2021a) and differs from 25-hydroxyvitamin D<sub>3</sub> (C<sub>27</sub>H<sub>44</sub>O<sub>2</sub>), the main metabolite of vitamin D<sub>3</sub> in the circulation, by a water molecule (Figure 1). Calcidiol monohydrate and 25-hydroxyvitamin D<sub>3</sub> are known to be functionally equal and are often used as synonymous.

<sup>10</sup> 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<sub>2</sub> and D<sub>3</sub>): 1 µg = 40 IU and 0.025 µg = 1 IU.





**Figure 1:** Chemistry of vitamin D

Ergocalciferol (a), cholecalciferol (b), calcidiol monohydrate (c), and 25-hydroxyvitamin D<sub>3</sub> (d). Source: PubChem (CID 5280793, 5280795, 6441383 and 5283731)<sup>11</sup>

Authorised forms of vitamin D for addition to foods<sup>2</sup> and for use in food supplements<sup>1</sup> 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

|                 | <b>Addition to foods Regulation (EC) 1925/2006<sup>2</sup></b> | <b>Food supplements Directive 2002/46/EC<sup>1</sup></b> |
|-----------------|--|--|
| Cholecalciferol | X  | X  |
| Ergocalciferol  | X  | X  |

<sup>11</sup> <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>.

## 3.2. Physiology and metabolism

### 3.2.1. Cutaneous synthesis

Vitamin D<sub>3</sub> 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<sub>3</sub> in the upper layers of the skin. Immediately after its formation, previtamin D<sub>3</sub> thermally isomerises to vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>2</sub>, vitamin D<sub>3</sub> or calcidiol would influence dermal synthesis of vitamin D<sub>3</sub>, 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<sub>2</sub> and vitamin D<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> (Thompson et al., 1966; Lo et al., 1985; Jones, 2014; Borel et al., 2015; Reboul, 2015; EFSA NDA Panel, 2018). Absorption of vitamins D<sub>2</sub> and D<sub>3</sub> occurs mostly in the distal small intestine and is dependent on the presence of bile acids and micelle formation. Vitamins D<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> or D<sub>3</sub>. 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<sub>2</sub> and D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> (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<sub>3</sub> from dermal synthesis is transported in plasma bound to the specific vitamin D-binding protein (DBP), whereas dietary vitamins D<sub>2</sub> and D<sub>3</sub> (from food and supplements) are transported in chylomicrons, with some transfer to DBP. 25(OH)D resulting from hydroxylation of vitamins D<sub>2</sub> and D<sub>3</sub> 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)<sub>2</sub>D, which is primarily transported bound to DBP and to a lesser extent to albumin (EFSA NDA Panel, 2016).



### 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)<sub>2</sub>D 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)<sub>2</sub>D, 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<sub>2</sub> supplementation, vitamin D was found in adipocyte lipid droplets as both vitamin D<sub>3</sub> and its metabolites (25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>) (Malmberg et al., 2014). About 75% of vitamin D<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> or vitamin D<sub>3</sub> (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)<sub>2</sub>D concentrations than with 'normal' serum 1,25(OH)<sub>2</sub>D 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 1 $\alpha$ -hydroxylation of 25(OH)D to 1,25(OH)<sub>2</sub>D by a P450 enzyme, the 25-hydroxyvitamin D 1 $\alpha$ -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)<sub>2</sub>D with a systemic action, whereas in other organs, such as bone, the parathyroid glands, and the placenta, the synthesis of 1,25(OH)<sub>2</sub>D 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)<sub>2</sub>D (Jones, 2014; EFSA NDA Panel, 2016).

Only 1,25(OH)<sub>2</sub>D produced in the kidney reaches the bloodstream, in which it is transported bound to DBP and then released as free 1,25(OH)<sub>2</sub>D, 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)<sub>2</sub>D may also activate nongenomic pathways (Bouillon et al., 2019). Although vitamins D<sub>2</sub> and D<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub> (Durrant et al., 2022).

25(OH)D and 1,25(OH)<sub>2</sub>D are inactivated, primarily in the kidney, through 24-hydroxylation (C24 hydroxylation pathway), resulting in 24,25(OH)<sub>2</sub>D and 1,24,25-trihydroxyvitamin D (1,24,25(OH)<sub>3</sub>D), respectively. 24,25(OH)<sub>2</sub>D prevents conversion of 25(OH)D to 1,25(OH)<sub>2</sub>D, whereas 1,24,25(OH)<sub>3</sub>D leads to calcitric acid (EFSA NDA Panel, 2016). The enzyme 25(OH)D-24 hydroxylase (CYP24A1) is reciprocally regulated (stimulated by 1,25(OH)<sub>2</sub>D<sub>3</sub> and suppressed by PTH), which tends to sustain blood 1,25(OH)<sub>2</sub>D<sub>3</sub> 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)<sub>2</sub>D leading, in sequential steps, to 25(OH)D-26,23-lactone and 1,25(OH)<sub>2</sub>D-26,23-lactone, respectively (Jones, 2014; Jones et al., 2014).

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)<sub>2</sub>D could have biologic effects different from 1,25(OH)<sub>2</sub>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. calcitric 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<sub>2</sub> is of dietary origin only, while serum 25(OH)D<sub>3</sub> may be of dietary or dermal origin. Serum 25(OH)D has a long mean half-life of ~ 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<sub>3</sub> and 25(OH)D<sub>2</sub> separately. LC–MS/MS can also measure other vitamin D metabolites, such as 24,25(OH)<sub>2</sub>D or 3-epi-25-hydroxyvitamin D<sub>3</sub> (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)<sup>12</sup> 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<sub>2</sub> and 25(OH)D<sub>3</sub>). 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 ≤ 10% CV and 9 out of 12 were ≤ ±5% mean bias), 10 out of 12 showed changes in response in the eight samples with high concentrations of 25(OH)D<sub>2</sub> (> 30 nmol/L), suggesting that response or recovery for 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> 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).

<sup>12</sup> 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.

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

Increasing oral vitamin D<sub>2</sub> and D<sub>3</sub> 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<sub>3</sub> raised 25(OH)D concentrations more than vitamin D<sub>2</sub>, 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<sub>2</sub> and D<sub>3</sub>) 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<sub>2</sub> supplements are less effective in raising or maintaining serum 25(OH)D concentrations compared to vitamin D<sub>3</sub> (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<sub>2</sub> or vitamin D<sub>3</sub> increased serum 25(OH)D concentration in the same way as the corresponding supplement, but the increment was lower with vitamin D<sub>2</sub> than with vitamin D<sub>3</sub> (Natri et al., 2006; Tripkovic et al., 2017). In a systematic review based on 24 intervention studies, vitamin D<sub>3</sub> was found to be more efficient in increasing both total 25(OH)D and 25(OH)D<sub>3</sub> concentrations, and in regulating PTH concentrations, than vitamin D<sub>2</sub> 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<sub>3</sub> may be only marginally better than vitamin D<sub>2</sub> 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<sub>3</sub> may be the preferred substrate for hepatic conversion to 25(OH)D (Holmberg et al., 1986; Tripkovic et al., 2012), and that vitamin D<sub>3</sub> and its metabolites have higher binding affinity to DBP as compared to vitamin D<sub>2</sub> (Houghton and Vieth, 2006). In addition, toxicity and repletion studies suggest some preferential non-specific catabolism of vitamin D<sub>2</sub> compared to vitamin D<sub>3</sub>, accelerating its degradation, especially at 'high' doses (Jones, 2013; EFSA NDA Panel, 2018). No statistically significant differences between the effects of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> 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<sub>2</sub>, either in fortified foods or supplements, decreases serum 25(OH)D<sub>3</sub> concentrations to levels lower than those found in the placebo group, indicating possible replacement of serum 25(OH)D<sub>3</sub> by serum 25(OH)D<sub>2</sub> 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<sub>3</sub>, 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

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<sub>3</sub> 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<sub>3</sub>, 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



response to vitamin D supplementation across trials (Hyppönen 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<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> 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 1 $\alpha$ -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<sub>3</sub>-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  $\mu$ g/day vitamin D<sub>3</sub> 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)<sub>2</sub>D<sub>3</sub> 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)<sub>2</sub>D<sub>3</sub> 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).

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<sub>3</sub> 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)<sub>2</sub>D by 1 $\alpha$ -hydroxylation. Its potential as a biomarker for vitamin D status remains to be established (EFSA NDA Panel, 2016).

1,25(OH)<sub>2</sub>D 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)<sub>2</sub>D 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<sub>3</sub>

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<sub>3</sub> 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  $\mu$ g/week (21.43  $\mu$ g/day) of calcidiol monohydrate or vitamin D<sub>3</sub>, 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<sub>3</sub> 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<sub>3</sub> on serum PTH. In the second RCT (Jakobsen et al., 2017), young male adults living in Denmark consumed 10  $\mu$ g/day of vitamin D<sub>2</sub>, vitamin D<sub>3</sub> or calcidiol for 6 weeks each following a randomised cross-over design, with no washout period. Mean achieved serum 25(OH)D

concentrations were approximately 10 nmol/L higher with calcidiol compared to vitamin D<sub>3</sub> (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<sub>2</sub>, 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<sub>3</sub> 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<sub>3</sub>. The same doses of calcidiol and vitamin D<sub>3</sub> 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<sub>3</sub> (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<sub>3</sub> arms were combined by the authors. In another RCT (Navarro-Valverde et al., 2016), vitamin D<sub>3</sub> 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<sub>3</sub>) 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<sub>3</sub> on serum 25(OH)D concentrations

The achieved (end-of-trial) serum 25(OH)D concentrations were higher with calcidiol than with vitamin D<sub>3</sub>, except when calcidiol doses were much lower (i.e., 5 and 7 µg/day) than vitamin D<sub>3</sub> 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<sub>3</sub> (Cashman et al., 2012; Navarro-Valverde

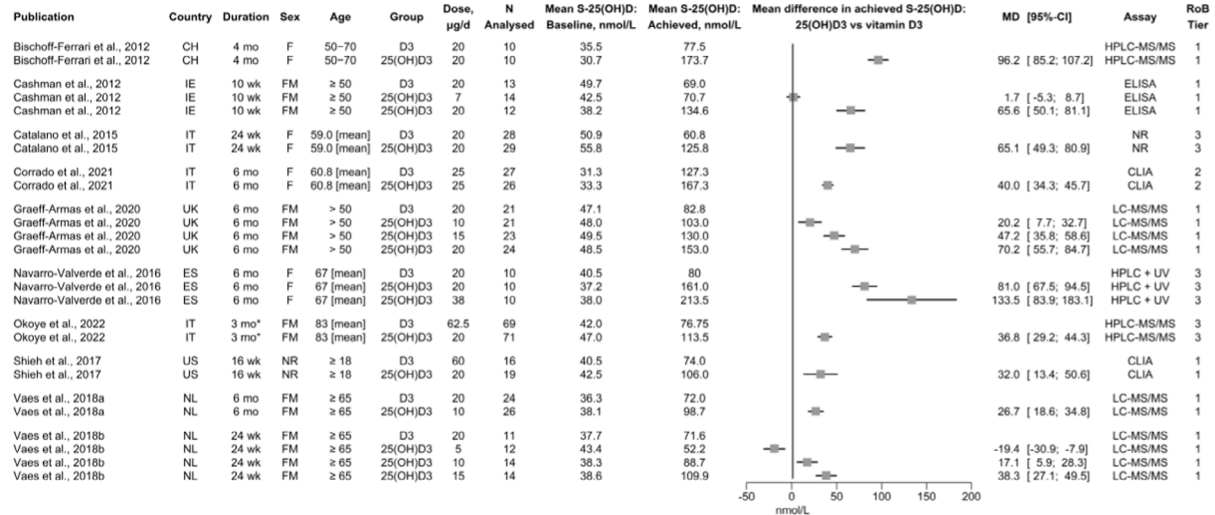


et al., 2016; Vaes et al., 2018b; Graeff-Armas et al., 2020), the achieved serum 25(OH)D concentrations (absolute values) were higher for calcidiol as compared to vitamin D<sub>3</sub> and increased with increasing doses of calcidiol.

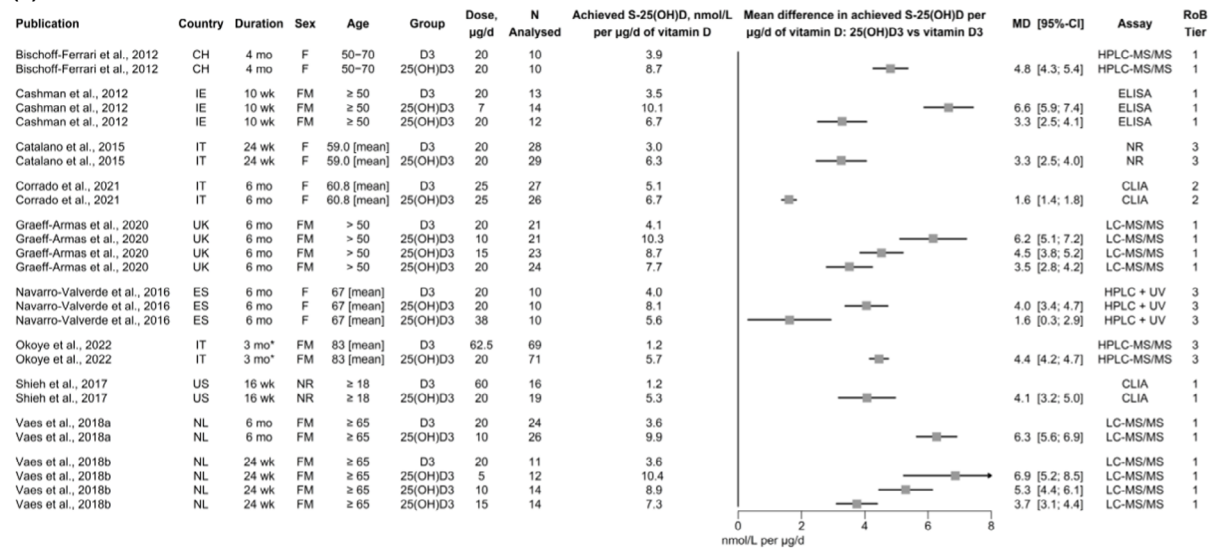
Owing to the disparity of vitamin D<sub>3</sub> and calcidiol doses used within and across studies, the mean difference in the achieved serum 25(OH)D concentrations per µg/day between calcidiol and vitamin D<sub>3</sub> (reference) was calculated. In all RCTs, the achieved serum 25(OH)D concentrations per µg/day of vitamin D were higher with calcidiol than with vitamin D<sub>3</sub>, although mean differences between calcidiol and vitamin D<sub>3</sub> 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<sub>3</sub>, the ratio of mean achieved serum 25(OH)D concentrations per µg/day between calcidiol and vitamin D<sub>3</sub> (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<sub>3</sub> used as reference. When 20 µg/day of vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> intake and serum 25(OH)D at intakes above 30–50 µg/day (see Section 3.3.1.2).

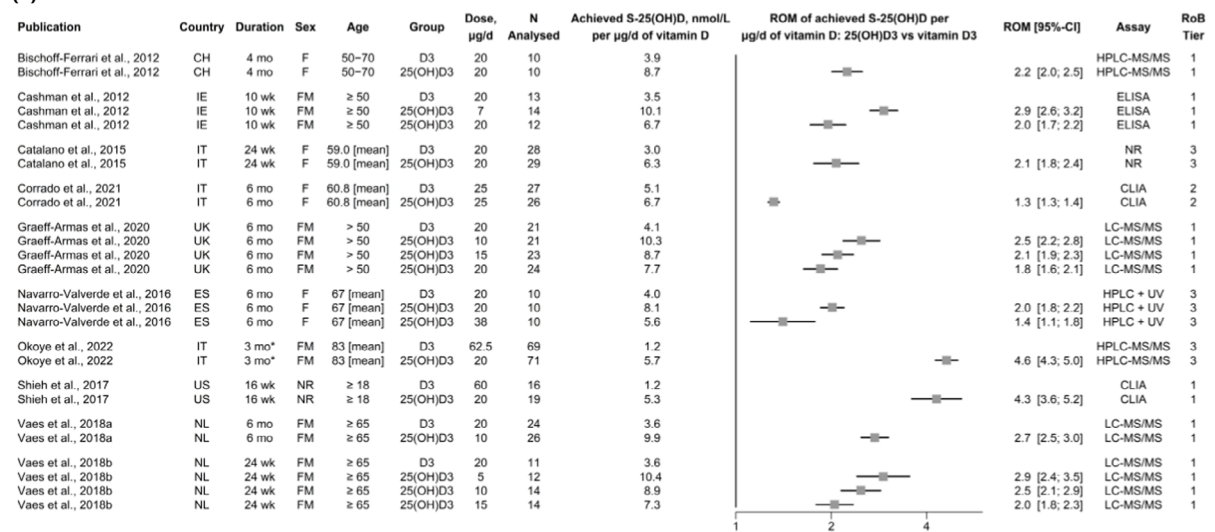
(a)



(b)



(c)



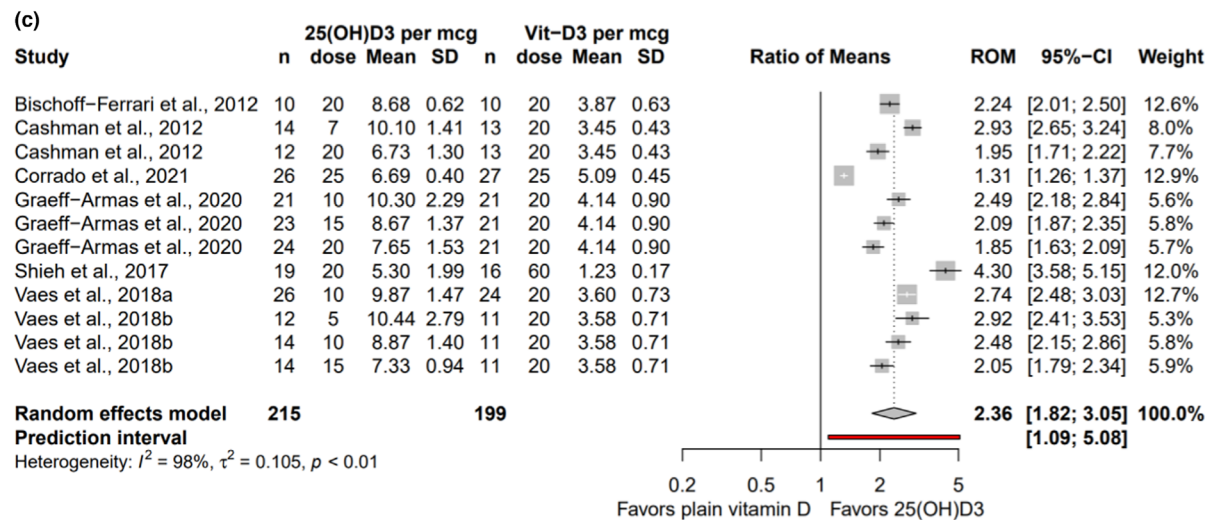
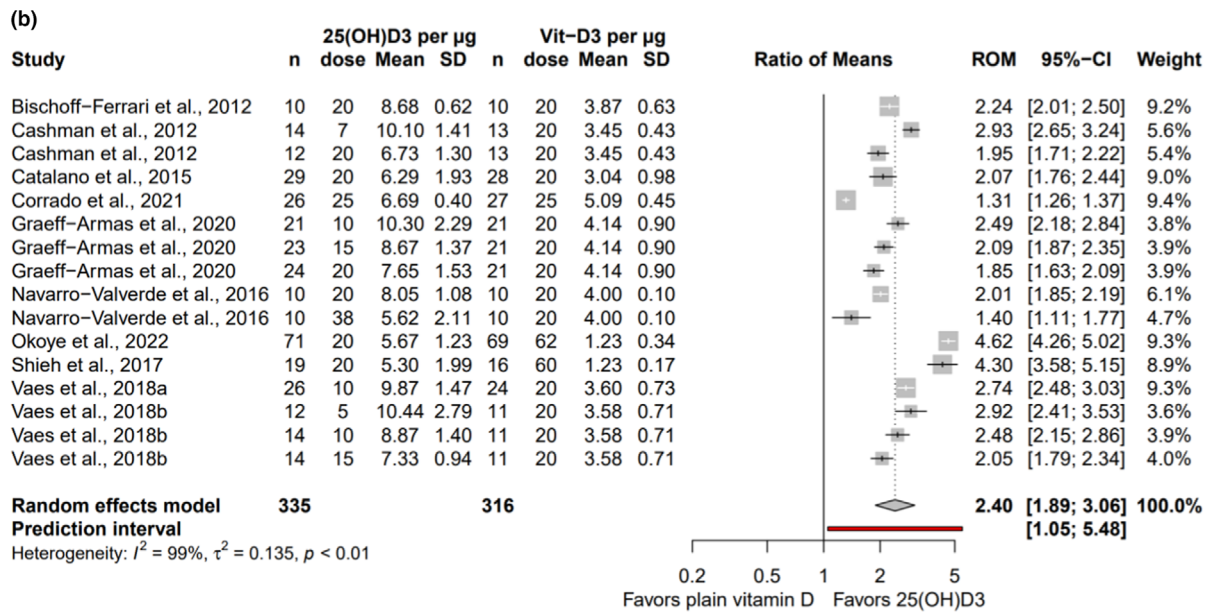
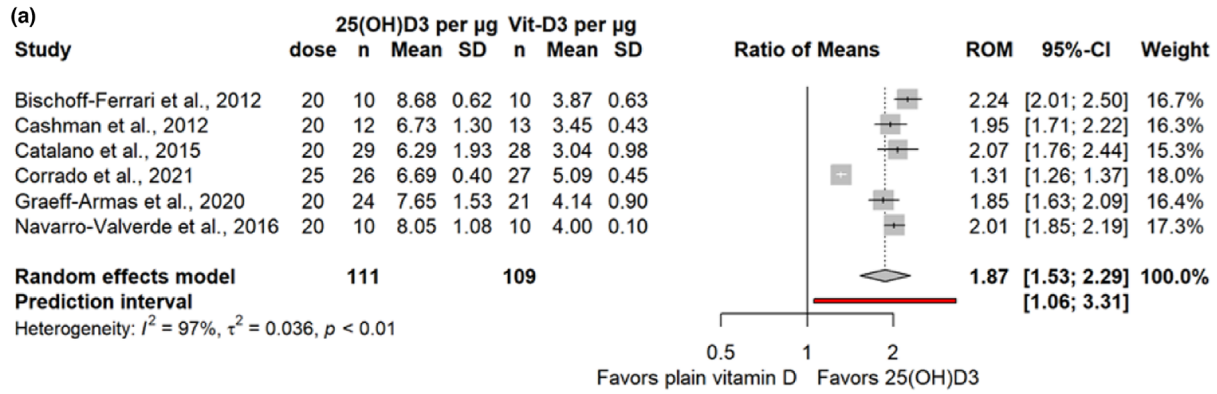
**Figure 2:** The effect of calcidiol monohydrate on serum 25(OH)D concentration compared to vitamin D<sub>3</sub>: (a) difference of means of achieved serum 25(OH)D concentration; (b) difference of means of achieved serum 25(OH)D concentration per µg/day of vitamin D; (c) ratio of means (ROM) of achieved 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<sub>3</sub>, (b) per µg/day of vitamin D, and (c) ratio of means. **Abbreviations:** CI, confidence interval; CH, Switzerland; CLIA, chemiluminescence immune assay; D<sub>3</sub>, vitamin D<sub>3</sub>; 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<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>, i.e., calcidiol. \*From hospital admission to 3 months after discharge.

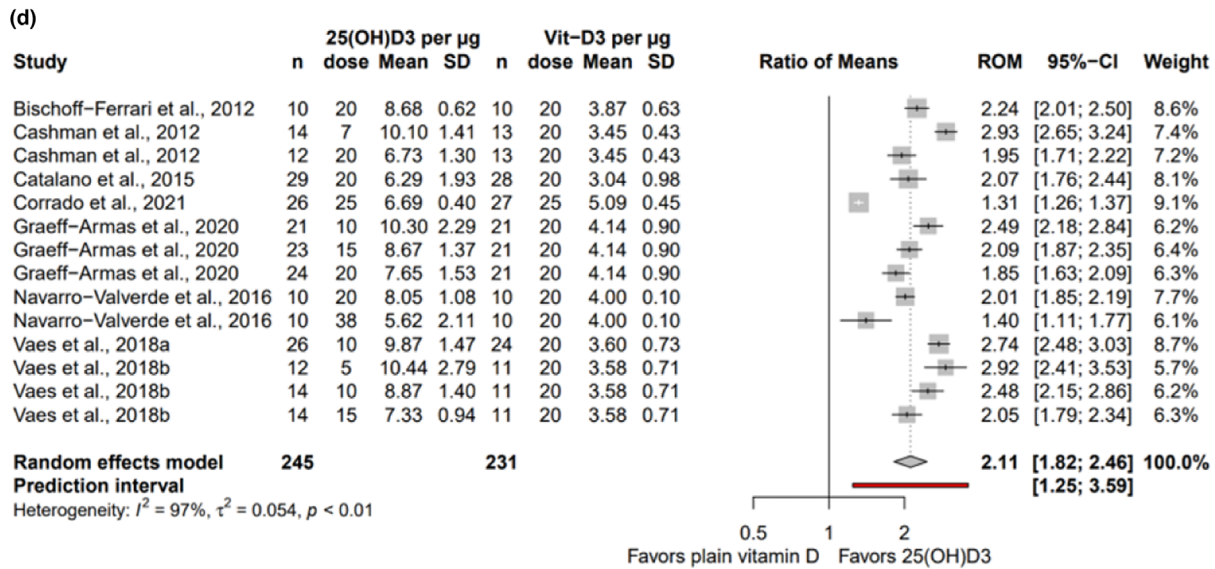
To control for the possible confounding introduced by differences in the dose of calcidiol and vitamin D<sub>3</sub> when investigating their relative bioavailability, the six RCTs that used the same doses were selected and meta-analysed using 25(OH)D concentrations as the outcome variable. Five RCTs 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<sub>3</sub> 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<sub>3</sub>. 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<sub>3</sub>. 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).







**Figure 3:** Relative bioavailability of calcidiol monohydrate [25(OH)D<sub>3</sub>] compared to vitamin D<sub>3</sub>: 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<sub>3</sub>; (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<sub>3</sub> 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<sub>3</sub>, vitamin D<sub>3</sub>; 25(OH)D<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>, i.e., calcidiol.

Since the dose–response curve between the intake of vitamin D<sub>3</sub> 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<sub>3</sub> as the reference dose (Figure 3d). The mean (95% CI) relative bioavailability of calcidiol compared to native vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub>). Doses of calcidiol ranged from 5 to 38 µg/day and doses of vitamin D<sub>3</sub> 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<sub>3</sub>) 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<sub>3</sub> 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<sub>3</sub>, 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.

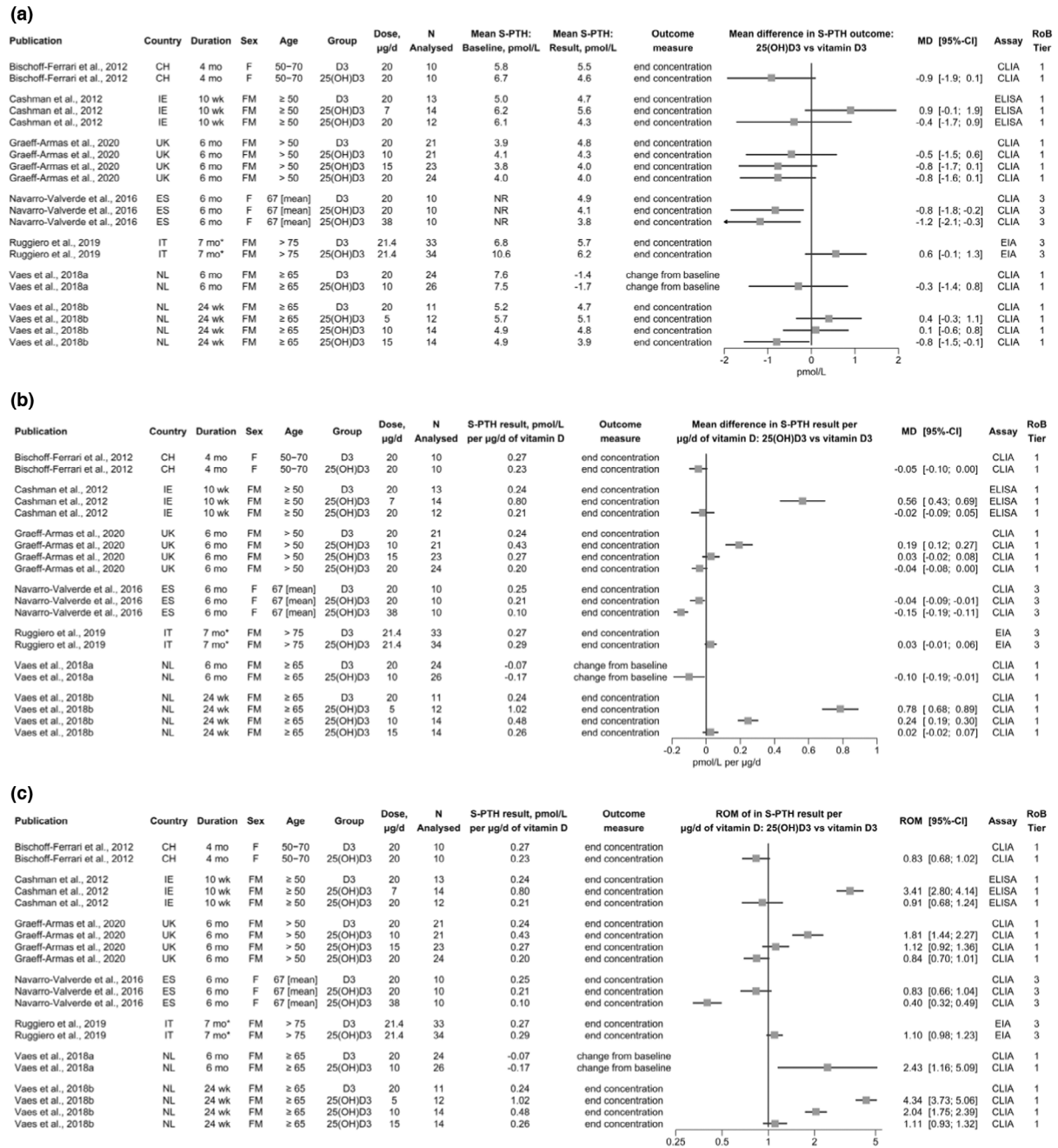
### 3.3.4.3. Effect of calcidiol monohydrate versus vitamin D<sub>3</sub> on serum PTH concentrations

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<sub>3</sub> (Figure 4a), except when calcidiol was administered at much lower doses (i.e., 5 and 7 µg/day) than vitamin D<sub>3</sub> (~ 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<sub>3</sub> (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<sub>3</sub> 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<sub>3</sub>, 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<sub>3</sub> 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<sub>3</sub> in suppressing PTH only when given at similar doses, the effect decreasing at lower doses of calcidiol.



**Figure 4:** The effect of calcidiol monohydrate on serum PTH concentration compared to vitamin D<sub>3</sub>: (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<sub>3</sub>. **Abbreviations:** CI, confidence interval; CH, Switzerland; CLIA, chemiluminescence immune assay; D<sub>3</sub>, vitamin D<sub>3</sub>; 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<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>, i.e., calcidiol.

\*From hospital admission to 7 months after discharge.



As discussed in Section 3.3.4.2, calcidiol raised serum 25(OH)D about twice as much than vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> and drops to 1.31 (95% CI: 1.26, 1.37) times at 25 µg/day (only 1 RCT available).

The Panel also notes that the mean relative bioavailability of calcidiol compared to vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub>) 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<sub>3</sub>) = 1 µg ergocalciferol (vitamin D<sub>2</sub>) = 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> used in the studies, and the bioequivalence of calcidiol versus vitamin D<sub>3</sub>.

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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub>

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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub>). 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<sub>2</sub>) and cholecalciferol (D<sub>3</sub>). Animal-derived foods such as fatty fish, fish offal, fish oil, and egg yolks are particularly rich in vitamin D<sub>3</sub>, whereas vitamin D<sub>2</sub> is mostly found in sources of plant origin, including some higher fungi such as mushrooms. Vitamin D<sub>2</sub> is produced in fungi by UV-B exposure of provitamin D<sub>2</sub> 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.<sup>13</sup> 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.<sup>14</sup>

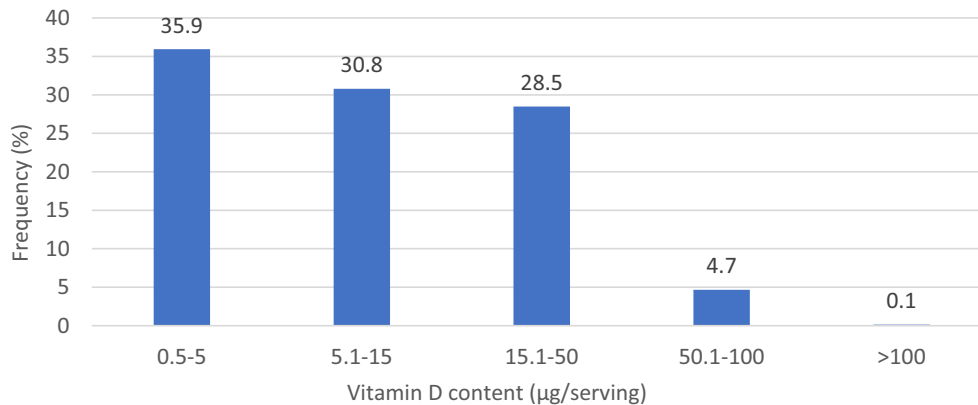
In the Mintel GNPD (from November 2017 to November 2022), a total of 6,169 packaged food products available in 24 EU Member States and Norway were identified as containing added vitamin D in the ingredients list. Most products belong to the Mintel categories 'dairy' (42%, includes dairy alternatives, 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).

<sup>13</sup> 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.

<sup>14</sup> Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p. 1 and Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children, OJ L 339, 6.12.2006, p. 16.

### Food supplements

In the EU, authorised forms of vitamin D for use in food supplements are ergocalciferol and cholecalciferol.<sup>15</sup> 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)

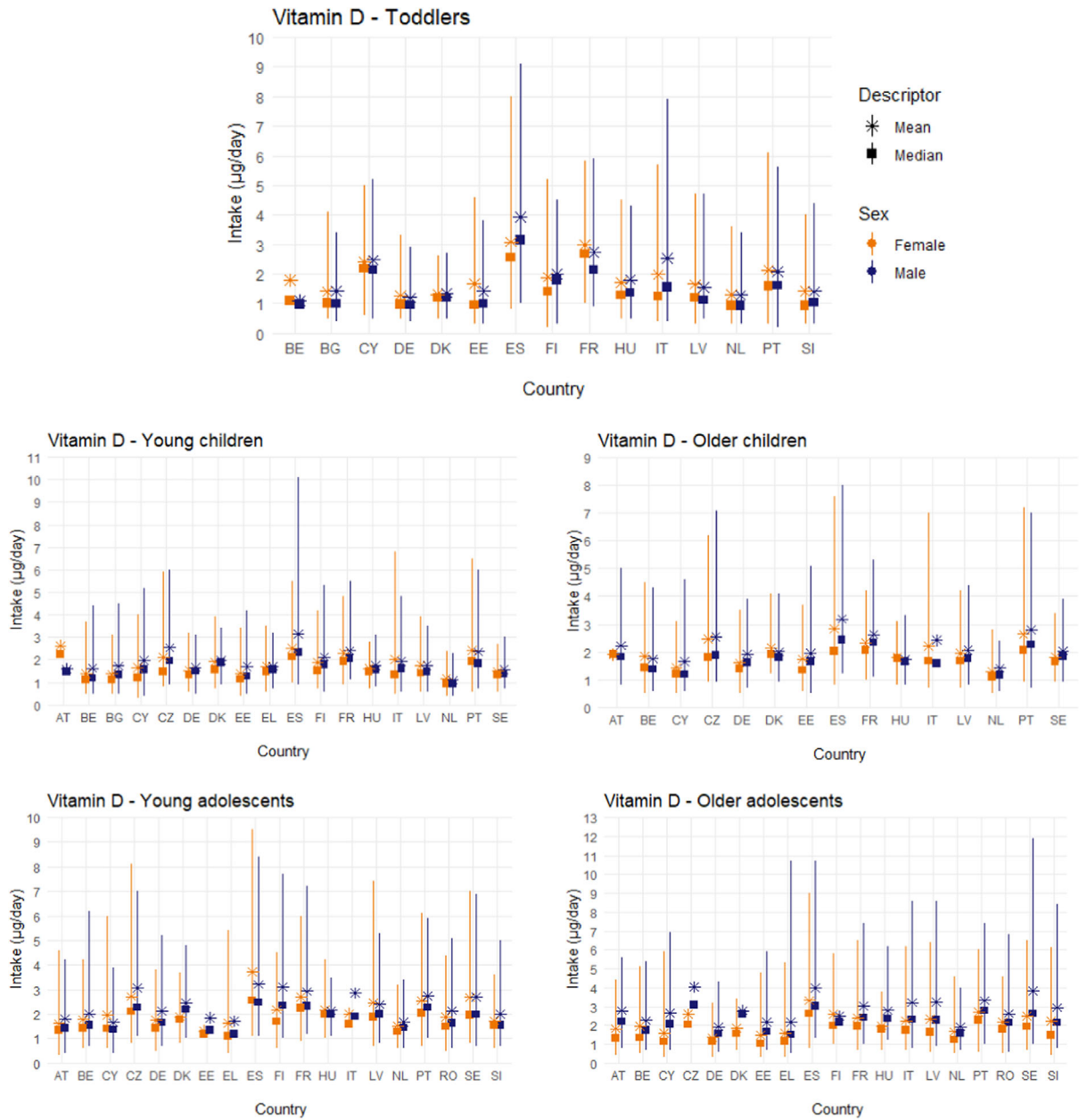
*Source:* Mintel GNPD. Search for vitamin D-containing supplements available in the EU market in the last 5 years (from November 2017 to November 2022). A total of 2,150 products available in 24 EU Member States and Norway were identified, of which 2,098 contained complete data on µg/serving.

### 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.

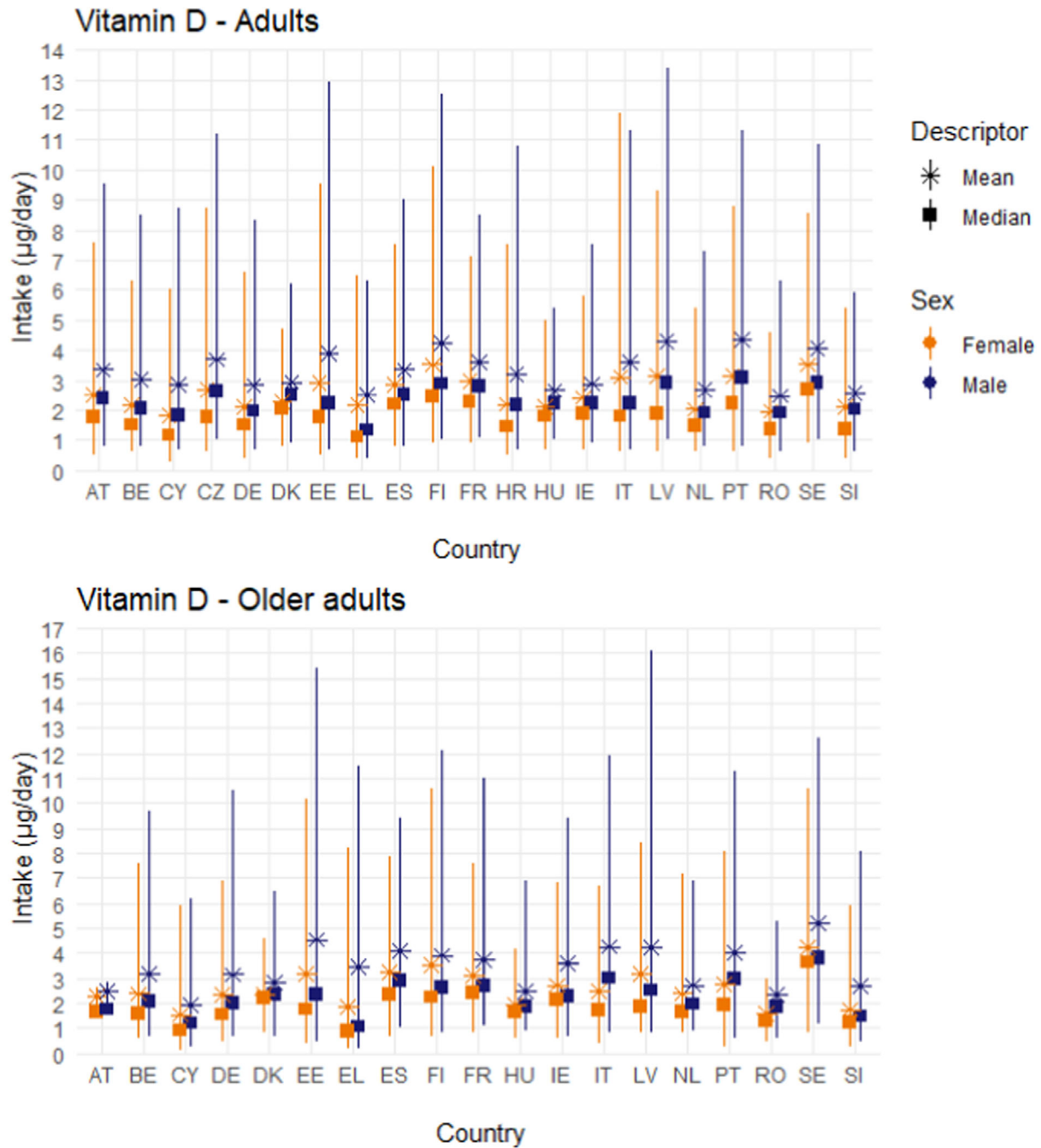
<sup>15</sup> Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p. 51.



**Figure 6:** Mean, median, 5th and 95th percentiles of background vitamin D intakes in toddlers ( $\geq 1$  year to  $< 3$  years old), young children ( $\geq 3$  years to  $< 7$  years old), older children ( $\geq 7$  years to  $< 10$  years old), intakes in young adolescents ( $\geq 10$  to  $< 14$  years) and older adolescents ( $\geq 14$  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.

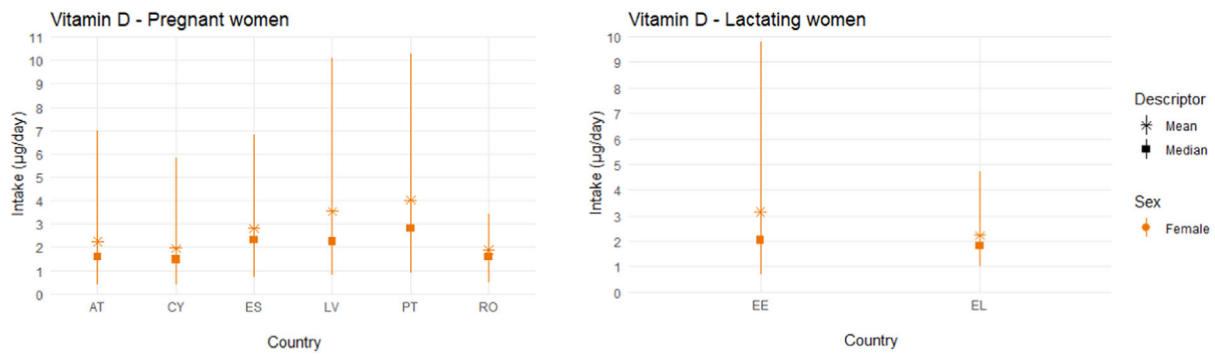


**Figure 7:** Mean, median, 5th and 95th percentiles of background vitamin D intakes in adults ( $\geq 18$  years to  $< 65$  years old) and older adults ( $\geq 65$  years), by sex and country.

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.





**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 <sup>(a)</sup>  |                     | Mean                |                     | p95 <sup>(a)</sup>  |                     |
|                                   |              | Min. <sup>(b)</sup> | Max. <sup>(b)</sup> | Min. <sup>(b)</sup> | Max. <sup>(b)</sup> | Min. <sup>(b)</sup> | Max. <sup>(b)</sup> | Min. <sup>(b)</sup> | Max. <sup>(b)</sup> |
| 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 <sup>(c)</sup>        | 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

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.

### *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).

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

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



### 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.

**Table 8:** Definitions for persistent hypercalcaemia and hypercalciuria cases<sup>(a)</sup>

| Persistent cases  | Transient cases   | Unclear cases   | Zero (persistent) cases   |
|---|---|---|---|
| <ul style="list-style-type: none"> <li>Serum/urine Ca was measured <i>in all participants</i> at least twice after baseline</li> </ul> <p>and either of the following:</p> <ul style="list-style-type: none"> <li>it was reported that retesting for elevated Ca concentration/ excretion was performed, i.e., the result was confirmed by retesting within a given timeand</li> <li>the elevated concentration/excretion persisted (NB: the participants were excluded from the original study if elevated concentration/excretion persisted after retesting)</li> </ul> <p><u>or</u></p> <ul style="list-style-type: none"> <li>the number of participants who developed recurrently elevated Ca concentration/excretion was reported</li> <li>i.e., Ca concentration/ excretion was elevated at least at two measurement points</li> <li>this might be despite the fact that retesting showed resolved concentrations/excretion</li> </ul> | <ul style="list-style-type: none"> <li>Serum/urine Ca was measured <i>in all participants</i> at least twice after baseline</li> </ul> <p>and either of the following:</p> <ul style="list-style-type: none"> <li>it was stated that retesting for elevated Ca concentration/ excretion was performed, i.e., the result was confirmed by retesting within a given timeand</li> <li>the elevated concentration/ excretion was resolved, and the participant continued in the studyor</li> <li>it was stated that the detected hypercalcaemia/ hypercalciuria case(s) was (were) transient</li> </ul> | <ul style="list-style-type: none"> <li>Serum/urine Ca was measured <i>in all participants</i> at least twice after baseline</li> </ul> <p>and</p> <ul style="list-style-type: none"> <li>it was unclear whether elevated Ca concentration/excretion persisted because <ul style="list-style-type: none"> <li>&gt; publications did not mention retesting for elevated Ca concentration/excretion</li> <li>&gt; 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</li> </ul> </li> </ul> | <ul style="list-style-type: none"> <li>serum/urine Ca was measured <i>in all participants</i> at least twice after baseline</li> </ul> <p>and either of the following:</p> <ul style="list-style-type: none"> <li>it was stated that no cases of hypercalcaemia/ hypercalciuria were detected during the entire intervention period</li> </ul> <p><u>or</u></p> <ul style="list-style-type: none"> <li>it was stated that Ca concentrations/excretion remained within the reference range in all participants throughout the intervention period</li> </ul> <p><u>or</u></p> <ul style="list-style-type: none"> <li>it was stated that the hypercalcaemia/ hypercalciuria cases were transient (please see the second column on the left for the definition)</li> </ul> |

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 ≤ 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

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<sub>2</sub> or D<sub>3</sub> 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  $\geq 100$  µg/day in adults and  $\geq 50$  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.

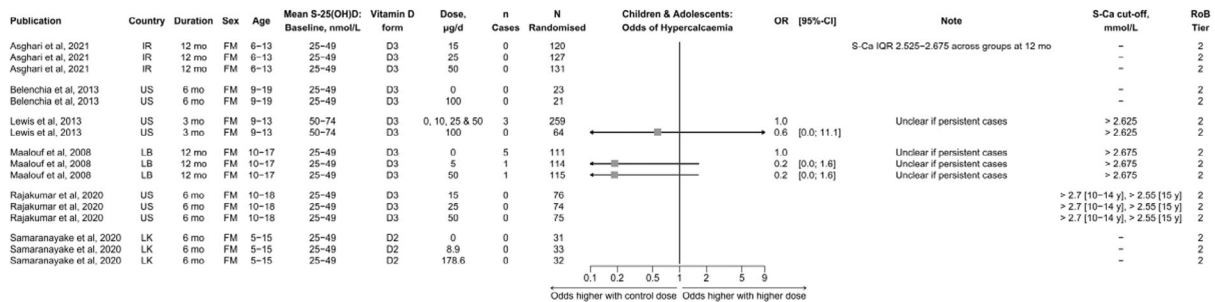
### 3.5.1.1. Children and adolescents

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<sub>3</sub> in five studies and vitamin D<sub>2</sub> 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  $\geq 50$  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<sub>3</sub> 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).



**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<sub>3</sub> supplements and one a combination of D<sub>2</sub> and D<sub>3</sub> (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.

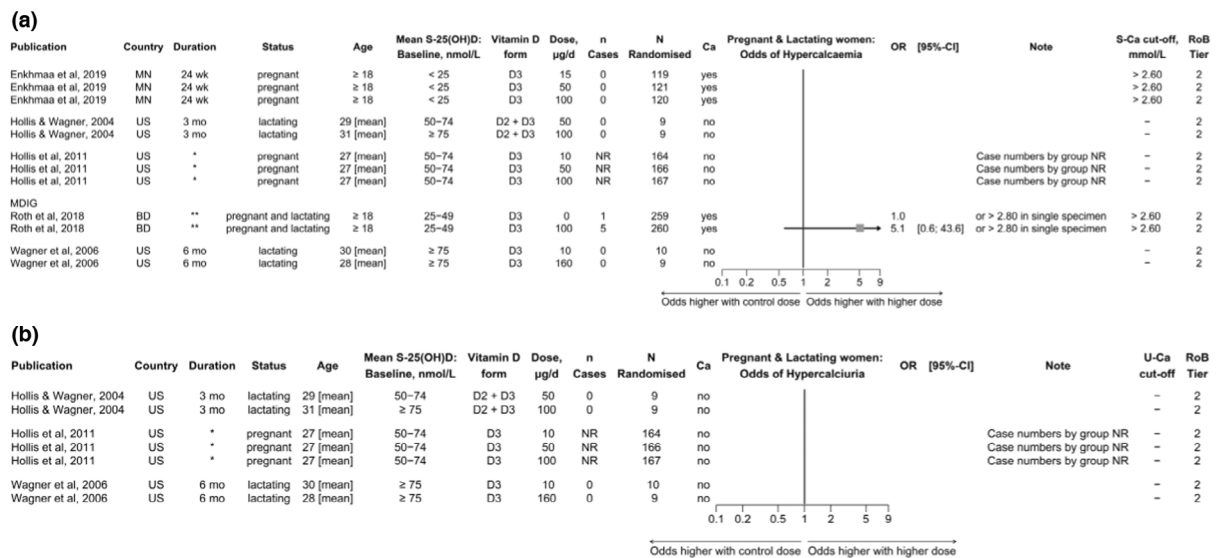
In Roth et al. (2018), no cases of hypercalcaemia were observed during the prenatal period. However, 5 women (1.9%) who received 100 µg/day vitamin D<sub>3</sub> and one woman (0.4%) in the placebo group developed hypercalcaemia postpartum (Figure 10a). In addition to the placebo and



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<sub>3</sub> 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).



**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<sub>2</sub> + 10 µg/day of vitamin D<sub>3</sub>) was compared with 50 µg/day of vitamin D (40 µg/day of vitamin D<sub>2</sub> + 10 µg/day of vitamin D<sub>3</sub>). 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',



n = 13;  $\geq 1$  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 $\leq 6$ 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  $\geq 75$  nmol/L in one RCT (Rorie et al., 2014).

Two RCTs administered vitamin D<sub>2</sub> and 11 gave vitamin D<sub>3</sub> as supplements. The highest vitamin D dose administered ranged from 100 to 275  $\mu\text{g}/\text{day}$  (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  $\mu\text{g}/\text{day}$  (Figure 11a). One RCT reported one transient case of hypercalcaemia in the arm supplemented with 100  $\mu\text{g}/\text{day}$  of vitamin D<sub>3</sub> 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  $\mu\text{g}/\text{day}$  vitamin D<sub>3</sub> 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  $\mu\text{g}/\text{day}$  of vitamin D<sub>3</sub> arm being just above the upper limit for hypercalcaemia on one occasion, which cannot be considered as persistent.

### *Studies with an intervention period $\geq 1$ 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  $\geq 75$  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<sub>2</sub> and D<sub>3</sub>, the remaining studies used vitamin D<sub>3</sub> supplements. Seven RCTs had used a calcium co-intervention. The highest vitamin D dose administered ranged from 100  $\mu\text{g}/\text{day}$  (Hin et al., 2016; Johnson et al., 2022) to 357  $\mu\text{g}/\text{day}$  (Rafii et al., 2019), and up to 2,500  $\mu\text{g}/\text{day}$  in an old study (Brohult and Jonson, 1973). The duration of the intervention ranged from 1 to 3 years.

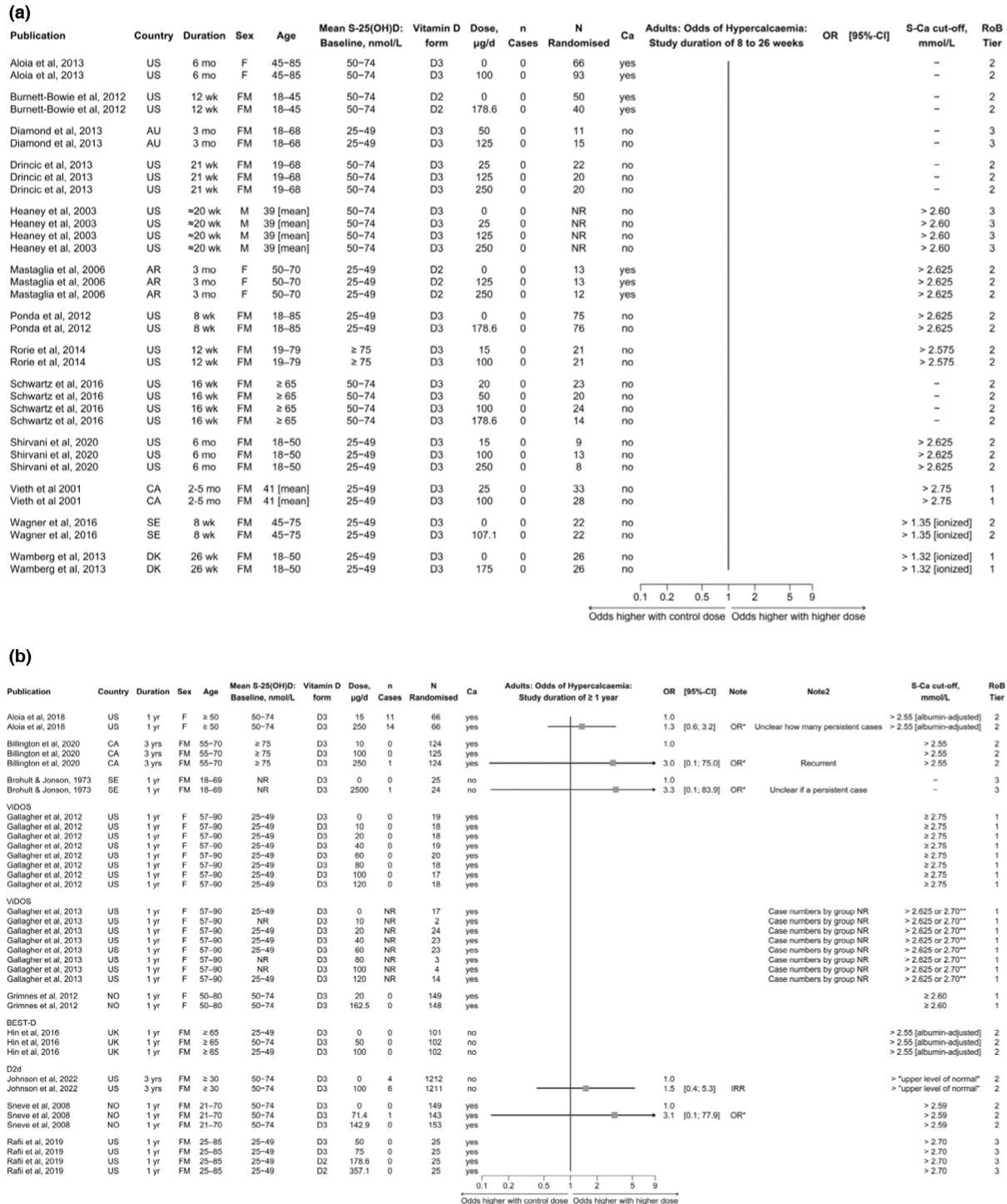
The criteria for defining hypercalcaemia varied across the RCTs. Cut-off points ranged between 2.55 and 2.8 mmol/L across the studies (n = 8). One study did not define hypercalcaemia (Brohult and Jonson, 1973). Johnson et al. (2022) defined hypercalcaemia as serum calcium levels 'greater than the site's 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.

Out of 10 RCTs, three reported cases of persistent hypercalcaemia with varying doses of vitamin D<sub>3</sub> (i.e., 71 to 250 µg/day), but the occurrence was rare and not specifically related to the dose of vitamin D<sub>3</sub> (Figure 11b). In a 3-year RCT (Johnson et al., 2022), daily supplementation with 100 µg of vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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 ≥ 75 nmol/L, Billington et al. (2020) found that one participant who received 250 µg/day of vitamin D<sub>3</sub> 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<sub>3</sub>, 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<sub>3</sub>, with no cases reported in the placebo group or the 143 µg/day vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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 ~ 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 ≥ 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).



**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

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<sub>3</sub> 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).

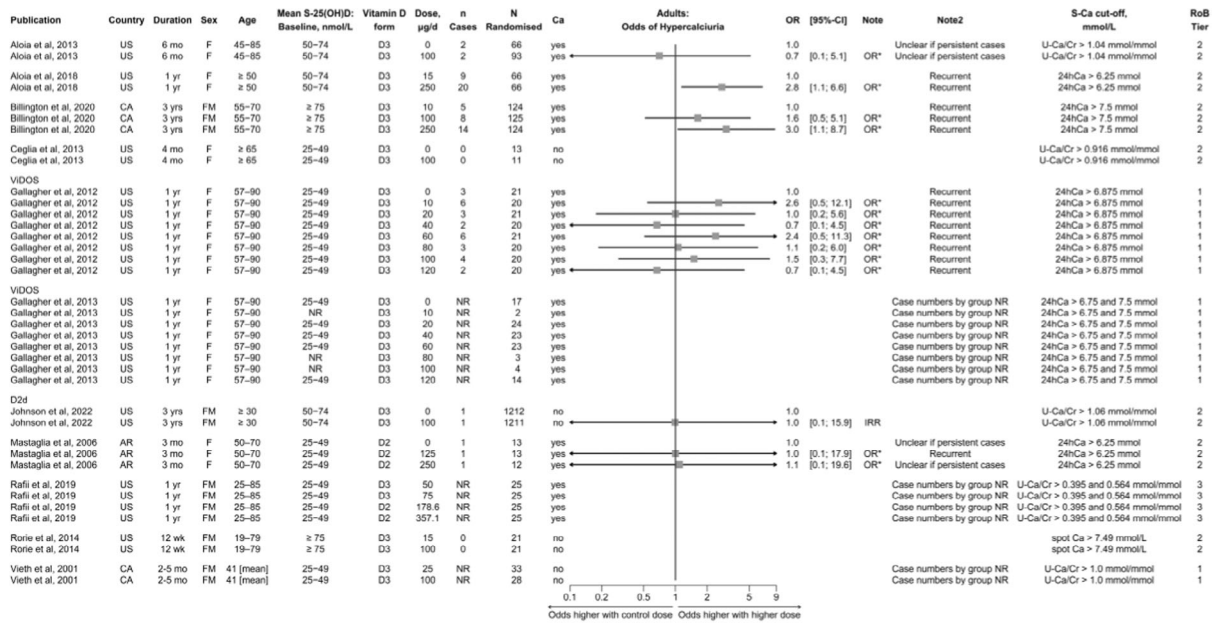
All the RCTs were conducted in America: eight in the USA (Gallagher et al., 2012; Aloia et al., 2013; Ceglia et al., 2013; Gallagher et al., 2013; Rorie et al., 2014; Aloia et al., 2018; Rafii et al., 2019; Johnson et al., 2022), two in Canada (Vieth et al., 2001; Billington et al., 2020) and one in 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<sub>2</sub> and the remaining vitamin D<sub>3</sub>. 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.





**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<sub>3</sub> and 75 µg/day of vitamin D<sub>2</sub>; 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<sub>3</sub> (Aloia et al., 2013), and with 125 and 250 µg/day of vitamin D<sub>2</sub> (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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> than with control doses during the



intervention period of 1 year (Aloia et al., 2018) or 3 years (Billington et al., 2020). In both RCTs vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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 ≈ 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<sub>2</sub> or D<sub>3</sub> 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<sub>3</sub> 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

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)<sub>2</sub>D 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)<sub>2</sub>D in the kidney, which in turn increases intestinal calcium absorption. Both PTH and 1,25(OH)<sub>2</sub>D 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)<sub>2</sub>D (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)<sub>2</sub>D 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<sub>3</sub>, 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)<sub>2</sub>D from DBP by 25(OH)D or other metabolites, which could promote the entry of free 1,25(OH)<sub>2</sub>D into target cells and bind the VDR (EFSA NDA Panel, 2018).

In general, serum total 25(OH)D concentrations ≥ 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)<sub>2</sub>D. 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)<sub>2</sub>D 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.

### 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<sub>3</sub> 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; Raffi 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:

- 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;
- 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;
- 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.

### 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 ≥ 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 ~ 57% of participants had serum 25(OH)D concentrations ≤ 40 nmol/L (Flicker et al., 2005).

The vitamin D form investigated was calcidiol in one study (Peacock et al., 2000), vitamin D<sub>2</sub> in two studies and vitamin D<sub>3</sub> in the remaining five studies. The highest vitamin D<sub>2</sub> or D<sub>3</sub> 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<sub>3</sub> 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.

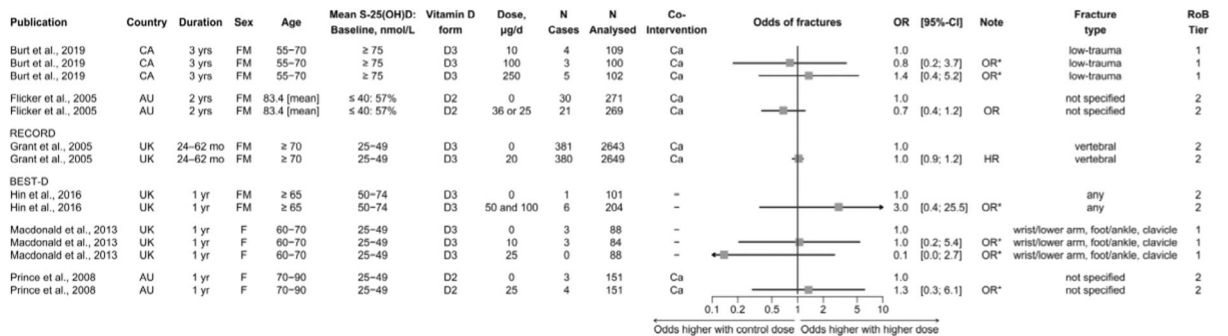
The available RCTs were very heterogeneous regarding how bone fractures were assessed and reported. Endpoints included low-trauma fractures, vertebral fractures, and any fracture, whereas in half of the RCTs bone fractures were not further specified. Some studies only reported fractures as an adverse 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.



Vitamin D<sub>2</sub> or D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> (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<sub>3</sub> (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<sub>2</sub> or vitamin D<sub>3</sub> 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, ≥ 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<sub>3</sub>. \*Effect size was computed based on the reported case numbers because pre-calculated effect sizes were not available.



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<sub>2</sub> in two studies and vitamin D<sub>3</sub> 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<sub>3</sub>. 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 approximately 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<sub>2</sub> or D<sub>3</sub> 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).

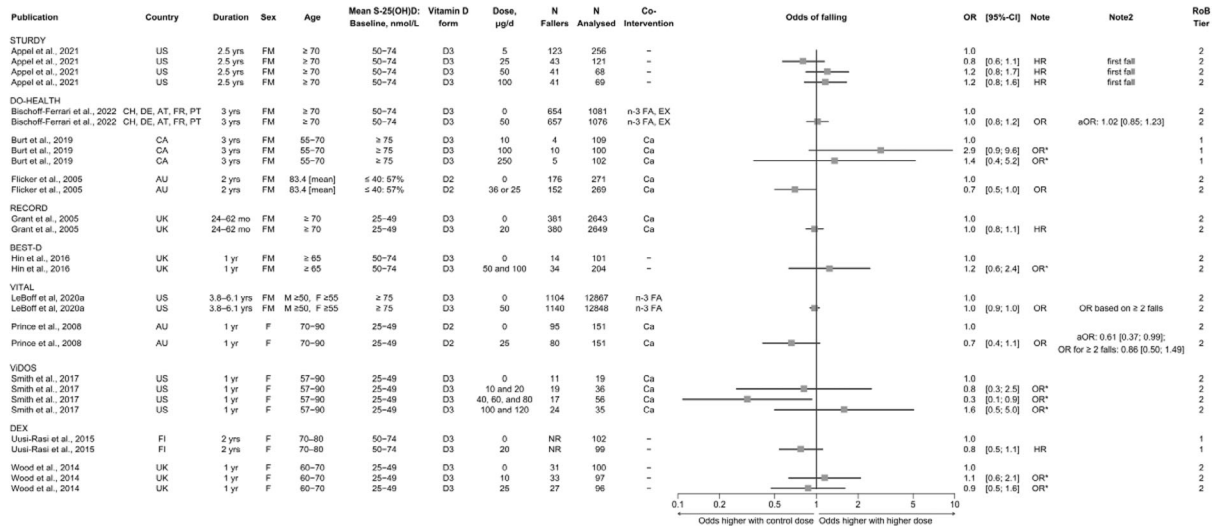
At doses of vitamin D<sub>3</sub> ≥ 100 µg/day, three RCTs (Smith et al., 2017; Burt et al., 2019; Appel et al., 2021) reported a marginally (not statistically significant) higher risk of falling at least once at high vitamin D doses versus control doses or placebo. In the RCT by Burt et al. (2019), which provided vitamin D<sub>3</sub> 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<sub>3</sub> 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).

In the 1-year RCT (Smith et al., 2017) 163 Caucasian overweight or obese postmenopausal women with mean serum 25(OH)D > 50 nmol/L and a relatively high history of falls in the previous year (~ 32%) were randomised to consume vitamin D<sub>3</sub> 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;  $p_{\text{adj}} = 0.063$ ), 3.15 (1.24–7.99;  $p_{\text{adj}} = 0.063$ ), and 5.63 (2.14–14.85;  $p_{\text{adj}} = 0.0027$ ), 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<sub>2</sub> or D<sub>3</sub> 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<sub>3</sub> 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).



**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<sub>3</sub> - 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<sub>3</sub> 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

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<sub>2</sub> or D<sub>3</sub> 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  $\geq 100$  µg/day in adults and  $\geq 50$  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. Approximately 20% of female participants were premenarcheal at study entry. Participants were divided into three groups receiving either placebo or vitamin D<sub>3</sub> 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<sub>3</sub> 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



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<sub>3</sub> 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).

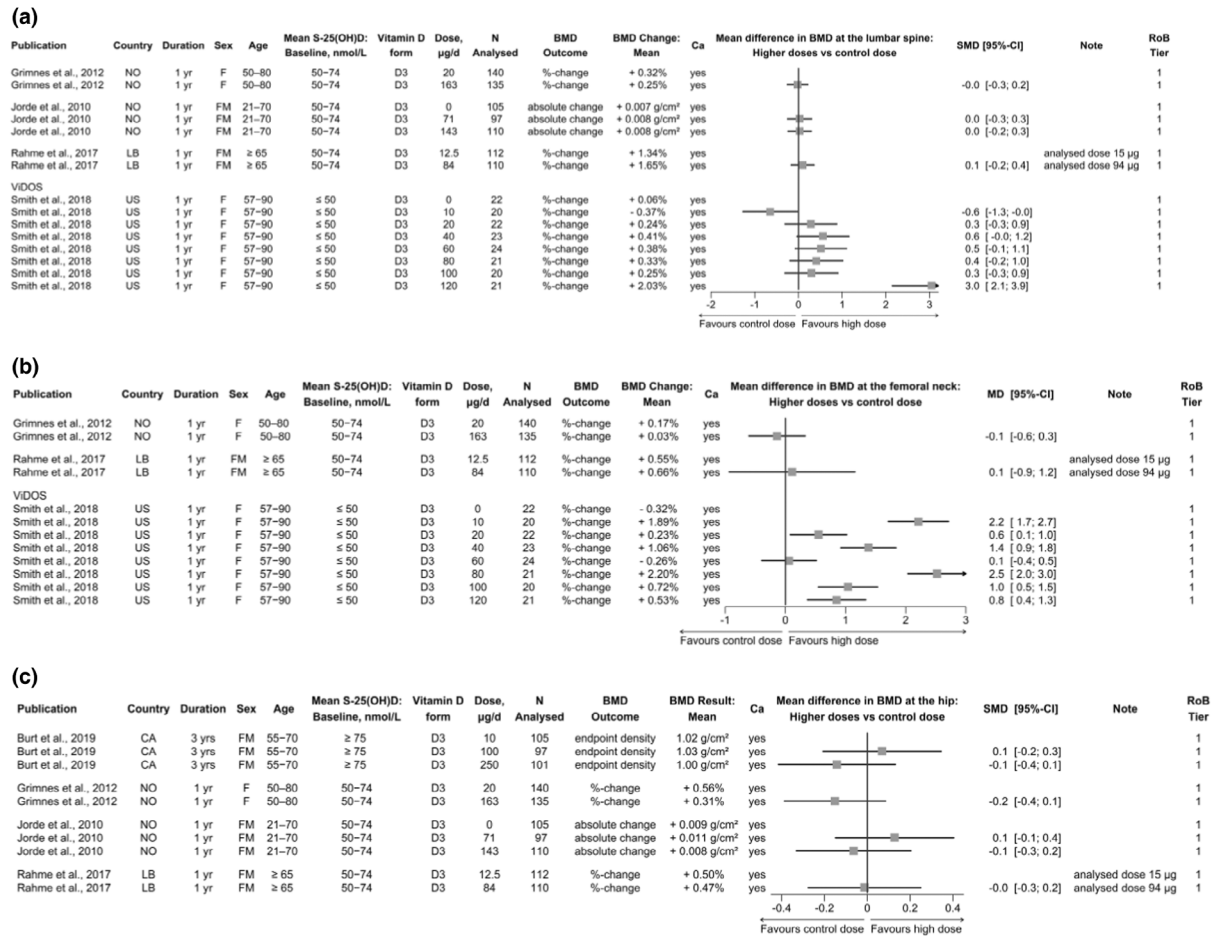
In the only RCT that assessed volumetric BMD using HR-pQCT (Burt et al., 2019), 311 males and females aged 55–70 years who were overweight on average and had serum 25(OH)D concentrations at baseline >75 nmol/L consumed vitamin D<sub>3</sub> supplements at doses of 10, 100 or 250 µg/day with calcium (to 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<sub>3</sub> and total volumetric BMD was found at both bone sites ( $p < 0.001$  for group  $\times$  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  $\times$  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.





**Figure 15:** The effect of high doses of vitamin D<sub>3</sub> 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,

12,500 µg of vitamin D<sub>3</sub> 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<sub>3</sub> (1,500 µg) or vitamin D<sub>3</sub> (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<sub>3</sub> 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<sub>3</sub> group, owing that high-dose vitamin D with extra calcium supplementation appears to be associated with increased levels of 1,25(OH)<sub>2</sub>D, 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)<sub>2</sub>D 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  $\geq 1$  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  $\leq 100$  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

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<sub>3</sub> 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<sub>2</sub> or D<sub>3</sub>) supplementation, with or without calcium, on kidney stones that excluded RCTs not controlling for calcium intake in the

placebo group have been published thereafter by the same authors. The first meta-analysis included trials lasting  $\geq 24$  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  $> 20 \mu\text{g}/\text{day}$  and 6 lasted  $\geq 1$  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<sub>2</sub> or D<sub>3</sub>  $\geq 70 \mu\text{g}/\text{day}$  and lasting  $\geq 1$  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)<sub>2</sub>D 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)<sub>2</sub>D 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)<sub>2</sub>D were associated to kidney stone formation, both circulating 25(OH)D and 1,25(OH)<sub>2</sub>D 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<sub>3</sub> was provided monthly at doses of 2,500  $\mu\text{g}$  ( $\sim 83 \mu\text{g}/\text{day}$ ) or 1,500  $\mu\text{g}$  ( $\sim 50 \mu\text{g}/\text{day}$ ) in the ViDA and D-health trials, respectively, or daily at doses of 50  $\mu\text{g}/\text{day}$  (VITAL) or 40 and 80  $\mu\text{g}/\text{day}$  (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  $\sim 75 \mu\text{g}/\text{day}$ . Achieved serum 25(OH)D concentrations  $\geq 100 \text{ nmol}/\text{L}$  were observed in trials with vitamin D supplementation at doses between 100  $\mu\text{g}$  and 300  $\mu\text{g}/\text{day}$ .

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<sub>2</sub> or D<sub>3</sub> and 5 RCTs investigated activated vitamin D analogues (calcitriol or ED-71). Baseline serum 25(OH)D concentration was  $< 50 \text{ nmol}/\text{L}$  in 6 RCTs,  $> 50 \text{ nmol}/\text{L}$  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



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 ≥ 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 (~ 50 µg/day) vitamin D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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 × 2 factorial design providing daily vitamin D<sub>3</sub> (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 ≥ 50 years and women aged ≥ 55 years, of which 5,106 African Americans. Vitamin D<sub>3</sub> 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.



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<sub>3</sub> supplementation on cancer incidence was found, even at serum 25(OH)D concentrations exceeding > 100 nmol/L, whereas vitamin D<sub>3</sub> 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)<sub>2</sub>D 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<sub>3</sub> 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<sub>3</sub> than in trials with vitamin D<sub>2</sub>. Again, there was no indication of higher mortality risk with higher vitamin D doses.

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

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<sub>3</sub>/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<sub>3</sub> arms. Two additional studies have been identified in which vitamin D<sub>3</sub> 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:

- hypercalciuria may precede hypercalcaemia, and none of the RCTs giving vitamin D alone at doses of 250 µg/day assessed urinary calcium;
- 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;
- 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 ≥ 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,

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<sub>2</sub> and D<sub>3</sub>), 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<sub>3</sub> 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<sub>3</sub> alone or in combination with vitamin D<sub>2</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>2</sub> 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<sub>3</sub> 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<sub>2</sub> and D<sub>3</sub>), and to calcidiol monohydrate, considering a CF for calcidiol monohydrate into vitamin D<sub>3</sub> 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



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<sub>3</sub> 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<sub>3</sub>) 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<sub>3</sub>) = 1 µg ergocalciferol (vitamin D<sub>2</sub>) = 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 <sup>(1)</sup>      | 25                                |
| 7–11 months <sup>(1),(2)</sup> | 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:

- To assess the relative bioavailability of equimolar doses of calcidiol monohydrate versus vitamin D<sub>3</sub> 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.
- To assess the relative bioavailability of equimolar doses of vitamin D<sub>2</sub> versus vitamin D<sub>3</sub> 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.
- 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.
- 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).
- To improve knowledge about the impact of the genetic background on the biological and clinical response to vitamin D supplementation (all forms).
- 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).



## References

- Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, Li Q, Weinstein SJ, Purdue M, Virtamo J, Horst R, Wheeler W, Chanock S, Hunter DJ, Hayes RB, Kraft P and Albanes D, 2010. Genome-wide association study of circulating vitamin D levels. *Human Molecular Genetics*, 19, 2739–2745. <https://doi.org/10.1093/hmg/ddq155>
- Akaike H, 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19, 716–723.
- Aloia JF, Talwar SA, Pollack S and Yeh J, 2005. A randomized controlled trial of vitamin D3 supplementation in African American women. *Archives of Internal Medicine*, 165, 1618–1623. <https://doi.org/10.1001/archinte.165.14.1618>
- Aloia JF, Dhaliwal R, Shieh A, Mikhail M, Islam S and Yeh JK, 2013. Calcium and vitamin d supplementation in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, 98, E1702–E1709.
- Aloia J, Dhaliwal R, Mikhail M, Shieh A, Stolberg A, Ragolia L, Fazzari M and Abrams SA, 2015. Free 25(OH)D and Calcium Absorption, PTH, and Markers of Bone Turnover. *The Journal of Clinical Endocrinology and Metabolism*, 100, 4140–4145. <https://doi.org/10.1210/jc.2015-2548>
- Aloia JF, Katumuluwa S, Stolberg A, Usera G, Mikhail M, Hoofnagle AN and Islam S, 2018. Safety of calcium and vitamin D supplements, a randomized controlled trial. *Clinical Endocrinology*, 89, 742–749. <https://doi.org/10.1111/cen.13848>
- Al-Shaar L, Nabulsi M, Maalouf J, El R, Vieth R, Beck TJ and El-Hajj FG, 2013. Effect of vitamin D replacement on hip structural geometry in adolescents: a randomized controlled trial. *Bone*, 56, 296–303. <https://doi.org/10.1016/j.bone.2013.06.020>
- Ammar M, Heni S, Tira MS, Khalij Y, Hamdouni H, Amor D, Ksibi S, Omezzine A and Bouslama A, 2022. Variability in response to vitamin D supplementation according to vitamin D metabolism related gene polymorphisms in healthy adults. *European Journal of Clinical Nutrition*, 77, 189–194. <https://doi.org/10.1038/s41430-022-01218-y>
- Andersen R, Molgaard C, Skovgaard LT, Brot C, Cashman KD, Jakobsen J, Lamberg-Allardt C and Ovesen L, 2008. Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-controlled intervention study. *British Journal of Nutrition*, 100, 197–207. <https://doi.org/10.1017/S000711450789430X>
- Appel LJ, Michos ED, Mitchell CM, Blackford AL, Sternberg AL, Miller ER, Juraschek SP, Schrack JA, Szanton SL, Charleston J, Minotti M, Baksh SN, Christenson RH, Coresh J, Drye LT, Guralnik JM, Kalyani RR, Plante TB, Shade DM, Roth DL, Tonascia J and STURDY Collaborative Research Group, 2021. The effects of four doses of vitamin D supplements on falls in older adults: a response-adaptive, randomized clinical trial. *Annals of Internal Medicine*, 174, 145–156. <https://doi.org/10.7326/m20-3812>
- Asghari G, Yuzbashian E, Wagner CL, Park Y, Mirmiran P and Hosseini F, 2021. Daily vitamin D<sub>3</sub> in overweight and obese children and adolescents: a randomized controlled trial. *European Journal of Nutrition*, 60, 2831–2840.
- Autier P, Gandini S and Mullie P, 2012. A systematic review: influence of vitamin D supplementation on serum 25-hydroxyvitamin D concentration. *The Journal of Clinical Endocrinology and Metabolism*, 97, 2606–2613. <https://doi.org/10.1210/jc.2012-1238>
- Avenell A, Mak JC and O'Connell D, 2014. Vitamin D and vitamin D analogues for preventing fractures in postmenopausal women and older men. *Cochrane Database of Systematic Reviews*, 2014, CD000227. <https://doi.org/10.1002/14651858.CD000227.pub4>
- Balachandar R, Pullakhandam R, Kulkarni B and Sachdev HS, 2021. Relative efficacy of vitamin D2 and vitamin D3 in improving vitamin D status: systematic review and meta-analysis. *Nutrients*, 13. <https://doi.org/10.3390/nu13103328>
- Bargagli M, Ferraro PM, Vittori M, Lombardi G, Gambaro G and Somani B, 2021. Calcium and vitamin D supplementation and their association with kidney stone disease: a narrative review. *Nutrients*, 13. <https://doi.org/10.3390/nu13124363>
- Barger-Lux MJ, Heaney RP, Dowell S, Chen TC and Holick MF, 1998. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporosis International*, 8, 222–230. <https://doi.org/10.1007/s001980050058>
- Belenchia AM, Tosh AK, Hillman LS and Peterson CA, 2013. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial. *American Journal of Clinical Nutrition*, 97, 774–781. <https://doi.org/10.3945/ajcn.112.050013>
- Benedik E, 2022. Sources of vitamin D for humans. *International Journal for Vitamin and Nutrition Research*, 92, 118–125. <https://doi.org/10.1024/0300-9831/a000733>
- Berry D and Hyppönen E, 2011. Determinants of vitamin D status: focus on genetic variations. *Current Opinion in Nephrology and Hypertension*, 20, 331–336. <https://doi.org/10.1097/MNH.0b013e328346d6ba>

- Bevilacqua G, Laskou F, Clynes MA, Jameson KA, Boucher BJ, Noonan K, Cooper C and Dennison EM, 2021. Determinants of circulating 25-hydroxyvitamin D concentration and its association with musculoskeletal health in midlife: findings from the Hertfordshire Cohort Study. *Metabolism Open*, 12, 100143. <https://doi.org/10.1016/j.metop.2021.100143>
- Bikle DD, 2021. Vitamin D: production, metabolism and mechanisms of action. *Endotext*, (Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., eds). South Dartmouth (MA): MDText.com, Inc. Copyright ©. MDText.com, Inc. pp. 2000–2022.
- Billington EO, Burt LA, Rose MS, Davison EM, Gaudet S, Kan M, Boyd SK and Hanley DA, 2020. Safety of high-dose vitamin D supplementation: secondary analysis of a randomized controlled trial. *Journal of Clinical Endocrinology and Metabolism*, 105, 1261–1273. <https://doi.org/10.1210/clinem/dgz212>
- Bischoff-Ferrari HA, Dawson-Hughes B, Stöcklin E, Sidelnikov E, Willett WC, Edel JO, Stähelin HB, Wolfram S, Jetter A, Schwager J, Henschkowski J, von Eckardstein A and Egli A, 2012. Oral supplementation with 25(OH) D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. *Journal of Bone and Mineral Research*, 27, 160–169. <https://doi.org/10.1002/jbmr.551>
- Bischoff-Ferrari HA, Dawson-Hughes B, Orav EJ, Staehelin HB, Meyer OW, Theiler R, Dick W, Willett WC and Egli A, 2016. Monthly high-dose vitamin D treatment for the prevention of functional decline: a randomized clinical trial. *JAMA Internal Medicine*, 176, 175–183. <https://doi.org/10.1001/jamainternmed.2015.7148>
- Bischoff-Ferrari HA, Vellas B, Rizzoli R, Kressig RW, da S JAP, Blauth M, Felson DT, McCloskey EV, Watzl B, Hofbauer LC, Minotti M, Baksh SN, Christenson RH, Coresh J, Drye LT, Guralnik JM, Kalyani RR, Plante TM, Shade DM, Roth DL, Tonascia J and STURDY Collaborative Research Group, 2020. Effect of vitamin D supplementation, omega-3 fatty acid supplementation, or a strength-training exercise program on clinical outcomes in older adults: the DO-HEALTH randomized clinical trial. *JAMA*, 324, 1855–1868. <https://doi.org/10.1001/jama.2020.16909>
- Bischoff-Ferrari HA, Freystatter G, Vellas B, Dawson HB, Kressig RW, Kanis JA, Willett WC, Manson JE, Rizzoli R, Theiler R, Hofbauer LC, Armbrecht G, da Silva JAP, Blauth M, de Godoi Rezende Costa MC, Lang W, Siebert U, Egli A, Orav EJ, Wiczorek M and DO-HEALTH Research Group, 2022. Effects of vitamin D, omega-3 fatty acids and a simple home strength exercise program on fall prevention: the DO-HEALTH randomized clinical trial. *American Journal of Clinical Nutrition*, 115, 1311–1321. <https://doi.org/10.1093/ajcn/nqac022>
- Bjelakovic G, Glud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelakovic M and Glud C, 2014. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database of Systematic Reviews*, Cd007470. <https://doi.org/10.1002/14651858.CD007470.pub3>
- Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, Saltzman E and Dawson-Hughes B, 2008. Vitamin D3 in fat tissue. *Endocrine*, 33, 90–94. <https://doi.org/10.1007/s12020-008-9051-4>
- Borel P, Caillaud D and Cano NJ, 2015. Vitamin D bioavailability: state of the art. *Critical Reviews in Food Science and Nutrition*, 55, 1193–1205. <https://doi.org/10.1080/10408398.2012.688897>
- Bouillon R, 2017. Genetic and Racial differences in the vitamin D endocrine system. *Endocrinology Metabolism Clinical North America*, 46, 1119–1135. <https://doi.org/10.1016/j.ecl.2017.07.014>
- Bouillon R and Carmeliet G, 2018. Vitamin D insufficiency: definition, diagnosis and management. *Best Practice & Research. Clinical Endocrinology & Metabolism*, 32, 669–684. <https://doi.org/10.1016/j.beem.2018.09.014>
- Bouillon R and Quesada Gomez JM, 2023. Comparison of calcifediol with vitamin D for prevention or cure of vitamin D deficiency. *The Journal of Steroid Biochemistry and Molecular Biology*, 228, 106248. <https://doi.org/10.1016/j.jsbmb.2023.106248>
- Bouillon R, Carmeliet G, Daci E, Segaert S and Verstuyf A, 1998. Vitamin D metabolism and action. *Osteoporosis International*, 8(Suppl. 2), S13–S19. <https://doi.org/10.1007/pl00022727>
- Bouillon R, Marcocci C, Carmeliet G, Bikle D, White JH, Dawson-Hughes B, Lips P, Munns CF, Lazaretti-Castro M, Giustina A and Bilezikian J, 2019. Skeletal and Extraskelatal Actions of Vitamin D: current Evidence and Outstanding Questions. *Endocrine Reviews*, 40, 1109–1151. <https://doi.org/10.1210/er.2018-00126>
- Breen ME, Laing EM, Hall DB, Hausman DB, Taylor RG, Isaacs CM, Ding KH, Pollock NK, Hamrick MW, Baile CA and Lewis RD, 2011. 25-hydroxyvitamin D, insulin-like growth factor-I, and bone mineral accrual during growth. *Journal of Clinical Endocrinology and Metabolism*, 96, E89–E98. <https://doi.org/10.1210/jc.2010-0595>
- Brohult J and Jonson B, 1973. Effects of large doses of calciferol on patients with rheumatoid arthritis. A double-blind clinical trial. *Scandinavian Journal of Rheumatology*, 2, 173–176.
- Burnett-Bowie SAM, Leder BZ, Henao MP, Baldwin CM, Hayden DL and Finkelstein JS, 2012. Randomized trial assessing the effects of ergocalciferol administration on circulating FGF23. *Clinical Journal of the American Society of Nephrology*, 7, 624–631. <https://doi.org/10.2215/CJN.10030911>
- Burt LA, Billington EO, Rose MS, Raymond DA, Hanley DA and Boyd SK, 2019. Effect of high-dose vitamin D supplementation on volumetric bone density and bone strength A randomized clinical trial. *JAMA-Journal of the American Medical Association*, 322, 736–745. <https://doi.org/10.1001/jama.2019.11889>
- Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, Makin HLJ, Pattni P, Sempos CT, Twomey P, Williams EL and Wise SA, 2018. Hydroxyvitamin D assays: an historical perspective from DEQAS. *The Journal of Steroid Biochemistry and Molecular Biology*, 177, 30–35. <https://doi.org/10.1016/j.jsbmb.2017.07.018>
- Cashman KD, 2012. The role of vitamins and dietary-based metabolites of vitamin D in prevention of vitamin D deficiency. *Food & Nutrition Research*, 56, 5383. <https://doi.org/10.3402/fnr.v56i0.5383>

- Cashman KD, Fitzgerald AP, Kiely M and Seamans KM, 2011. A systematic review and meta-regression analysis of the vitamin D intake-serum 25-hydroxyvitamin D relationship to inform European recommendations. *British Journal of Nutrition*, 106, 1638–1648. <https://doi.org/10.1017/S0007114511005058>
- Cashman KD, Seamans KM, Lucey AJ, Stöcklin E, Weber P, Kiely M and Hill TR, 2012. Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *American Journal of Clinical Nutrition*, 95, 1350–1356. <https://doi.org/10.3945/ajcn.111.031427>
- Cashman KD, Dowling KG, Skrabakova Z, Kiely M, Lamberg-Allardt C, Durazo-Arvizu RA, Sempos CT, Koskinen S, Lundqvist A, Sundvall J, Linneberg A, Thuesen B, Husemoen LL, Meyer HE, Holvik K, Gronborg IM, Tetens I and Andersen R, 2015. Standardizing serum 25-hydroxyvitamin D data from four Nordic population samples using the Vitamin D Standardization Program protocols: shedding new light on vitamin D status in Nordic individuals. *Scandinavian Journal of Clinical and Laboratory Investigation*, 75, 549–561. <https://doi.org/10.3109/00365513.2015.1057898>
- Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, Moreno L, Damsgaard CT, Michaelsen KF, Molgaard C, Jorde R, Grimnes G, Moschonis G, Mavrogianni C, Manios Y, Thamm M, Mensink GB, Rabenberg M, Busch MA, Cox L, Meadows S, Goldberg G, Prentice A, Dekker JM, Nijpels G, Pilz S, Swart KM, van Schoor NM, Lips P, Eiriksdottir G, Gudnason V, Cotch MF, Koskinen S, Lamberg-Allardt C, Durazo-Arvizu RA, Sempos CT and Kiely M, 2016. Vitamin D deficiency in Europe: pandemic? *American Journal of Clinical Nutrition*, 103, 1033–1044. <https://doi.org/10.3945/ajcn.115.120873>
- Cashman KD, Kiely ME, Andersen R, Gronborg IM, Tetens I, Tripkovic L, Lanham-New SA, Lamberg-Allardt C, Adebayo FA, Gallagher JC, Smith LM, Satchell JM, Huang Q, Ng K, Yuan C, Giovannucci EL, Rajakumar K, Patterson CG, Ohlund I, Lind T, Akeson PK and Ritz C, 2022a. Individual participant data (IPD)-level meta-analysis of randomised controlled trials to estimate the vitamin D dietary requirements in dark-skinned individuals resident at high latitude. *European Journal of Nutrition*, 61, 1015–1034. <https://doi.org/10.1007/s00394-021-02699-6>
- Cashman KD, Ritz C, Carlin A and Kennedy M, 2022b. Vitamin D biomarkers for dietary reference intake development in children: a systematic review and meta-analysis. *American Journal of Clinical Nutrition*, 115, 544–558. <https://doi.org/10.1093/ajcn/nqab357>
- Catalano A, Morabito N, Basile G, Cucinotta D and Lasco A, 2015. Calcifediol improves lipid profile in osteoporosis-treated postmenopausal women. *European Journal of Clinical Investigation*, 45, 144–149. <https://doi.org/10.1111/eci.12390>
- Ceglia L, Niramitmahapanya S, Da Silva MM, Rivas DA, Harris SS, Bischoff FH, Fielding RA and Dawson HB, 2013. A Randomized study on the effect of vitamin d3 supplementation on skeletal muscle morphology and vitamin d receptor concentration in older women. *Journal of Clinical Endocrinology and Metabolism*, 98, E1927–E1935. <https://doi.org/10.1210/jc.2013-2820>
- Cesareo R, Falchetti A, Attanasio R, Tabacco G, Naciu AM and Palermo A, 2019. Hypovitaminosis D: is it time to consider the use of calcifediol? *Nutrients*, 11. <https://doi.org/10.3390/nu11051016>
- Chatterjee R, Fuss P, Vickery EM, ES LB, Sheehan PR, Lewis MR, Dolor RJ, Johnson KC, Kashyap SR, Nelson J, Pittas AG and Group DdR, 2021. Vitamin D supplementation for prevention of cancer: the D2d cancer outcomes (D2dCA) ancillary study. *The Journal of Clinical Endocrinology and Metabolism*, 106, 2767–2778. <https://doi.org/10.1210/clinem/dgab153>
- Corrado A, Rotondo C, Cici D, Berardi S and Cantatore FP, 2021. Effects of different vitamin D supplementation schemes in post-menopausal women: a monocentric open-label randomized study. *Nutrients*, 13, 26. <https://doi.org/10.3390/nu13020380>
- Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, Atkinson S, Ward L, Moher D, Hanley D, Fang M, Yazdi F, Garrity C, Sampson M, Barrowman N, Tsertsvadze A and Mamaladze V, 2007. Effectiveness and safety of vitamin D in relation to bone health. *Evidence Report Technology Assess (Full Report)* 1–235.
- Crippa A, Discacciati A, Bottai M, Spiegelman D and Orsini N, 2019. One-stage dose–response meta-analysis for aggregated data. *Statistical Methods in Medical Research*, 28, 1579–1596. <https://doi.org/10.1177/0962280218773122>
- Darling AL, Blackburn DJ, Ahmadi KR and Lanham-New SA, 2021. Very high prevalence of 25-hydroxyvitamin D deficiency in 6433 UK South Asian adults: analysis of the UK Biobank Cohort. *British Journal of Nutrition*, 125, 448–459. <https://doi.org/10.1017/s0007114520002779>
- Dawson-Hughes B, Harris SS, Krall EA, Dallal GE, Falconer G and Green CL, 1995. Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. *American Journal of Clinical Nutrition*, 61, 1140–1145. <https://doi.org/10.1093/ajcn/61.4.1140>
- Dawson-Hughes B, Harris SS, Lichtenstein AH, Dolnikowski G, Palermo NJ and Rasmussen H, 2015. Dietary fat increases vitamin D-3 absorption. *Journal of Academic Nutrition Diet*, 115, 225–230. <https://doi.org/10.1016/j.jand.2014.09.014>
- de Jong MH, Nawijn EL and Verkaik-Kloosterman J, 2022. Contribution of fortified margarines and other plant-based fats to micronutrient intake in The Netherlands. *European Journal of Nutrition*, 61, 1893–1904. <https://doi.org/10.1007/s00394-021-02757-z>

- del Puente A, Esposito A, Savastano S, Carpinelli A, Postiglione L and Oriente P, 2002. Dietary calcium intake and serum vitamin D are major determinants of bone mass variations in women. A longitudinal study. *Aging Clinical and Experimental Research*, 14, 382–388.
- DerSimonian R and Laird N, 1986. Meta-analysis in clinical trials. *Controlled Clinical Trials*, 7, 177–188. [https://doi.org/10.1016/0197-2456\(86\)90046-2](https://doi.org/10.1016/0197-2456(86)90046-2)
- Diamond T, Wong YK and Golombick T, 2013. Effect of oral cholecalciferol 2,000 versus 5,000 IU on serum vitamin D, PTH, bone and muscle strength in patients with vitamin D deficiency. *Osteoporosis International*, 24, 1101–1105.
- Ding C, Parameswaran V, Blizzard L, Burgess J and Jones G, 2010. Not a simple fat-soluble vitamin: changes in serum 25-(OH)D levels are predicted by adiposity and adipocytokines in older adults. *Journal of Internal Medicine*, 268, 501–510. <https://doi.org/10.1111/j.1365-2796.2010.02267.x>
- Drincic A, Fuller E, Heaney RP and Armas LA, 2013. 25-Hydroxyvitamin D response to graded vitamin D3 supplementation among obese adults. *Journal of Clinical Endocrinology and Metabolism*, 98, 4845–4851.
- Dunlop E, Kiely ME, James AP, Singh T, Pham NM and Black LJ, 2021. Vitamin D food fortification and biofortification increases serum 25-hydroxyvitamin D concentrations in adults and children: an updated and extended systematic review and meta-analysis of randomized controlled trials. *Journal of Nutrition*, 151, 2622–2635. <https://doi.org/10.1093/jn/nxab180>
- Durazo-Arvizu RA, Dawson-Hughes B, Kramer H, Cao G, Merkel J, Coates PM and Sempos CT, 2017. The reverse J-shaped association between serum total 25-hydroxyvitamin D concentration and all-cause mortality: the impact of assay standardization. *American Journal of Epidemiology*, 185, 720–726. <https://doi.org/10.1093/aje/kww244>
- Durrant LR, Bucca G, Hesketh A, Möller-Levet C, Tripkovic L, Wu H, Hart KH, Mathers JC, Elliott RM, Lanham-New SA and Smith CP, 2022. Vitamins D(2) and D(3) have overlapping but different effects on the human immune system revealed through analysis of the blood transcriptome. *Frontiers of Immunology*, 13, 790444. <https://doi.org/10.3389/fimmu.2022.790444>
- Durup D, Jorgensen HL, Christensen J, Schwarz P, Heegaard AM and Lind B, 2012. A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice: the CopD study. *The Journal of Clinical Endocrinology and Metabolism*, 97, 2644–2652. <https://doi.org/10.1210/jc.2012-1176>
- EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal* 2010;8(6):1637, 90 pp. <https://doi.org/10.2903/j.efsa.2010.1637>
- EFSA (European Food Safety Authority), 2011a. Methodological characteristics of the national dietary surveys carried out in the European Union as included in the European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database. *Food additives & contaminants Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment*, 28, 975–995. <https://doi.org/10.1080/19440049.2011.576440>
- EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. *EFSA Journal* 2011;9(3):2097, 34 pp. <https://doi.org/10.2903/j.efsa.2011.2097>
- EFSA (European Food Safety Authority), 2015a. The food classification and description system FoodEx 2 (revision 2). *EFSA Supporting Publication* 2015;12(5):EN-804, 90 pp. <https://doi.org/10.2903/sp.efsa.2015.EN-804>
- EFSA (European Food Safety Authority), 2015b. Principles and process for dealing with data and evidence in scientific assessments. *EFSA Journal* 2015;13(5):4121, 35 pp. <https://doi.org/10.2903/j.efsa.2015.4121>
- EFSA (European Food Safety Authority), 2022. Protocol for the intake assessments performed in the context of the revision of Tolerable Upper Intake Levels for selected nutrients. *EFSA Supporting Publications* 2022;e200801E, 15 pp. <https://doi.org/10.2903/sp.efsa.2022.e200801>
- EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2018. Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources. *EFSA Journal* 2018;16(6):5294, 35 pp. <https://doi.org/10.2903/j.efsa.2018.5294>
- EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2005. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the evaluation of safety and efficacy of “Hy•D” (calcifediol), based on 25-hydroxycholecalciferol/25-hydroxy-pre-cholecalciferol, as feed additive in accordance with Council Directive 70/524/EEC. *EFSA Journal* 2005;3:224, 35 pp. <https://doi.org/10.2903/j.efsa.2005.224>
- EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2009. Safety and efficacy of 25-hydroxycholecalciferol as a feed additive for poultry and pigs. *EFSA Journal* 2009;7:969, 39 pp. <https://doi.org/10.2903/j.efsa.2009.969>
- EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2012. Scientific Opinion on the safety and efficacy of vitamin D3 (cholecalciferol) as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories, based on a dossier submitted by DSM. *EFSA Journal* 2012;10(12):2968, 26 pp. <https://doi.org/10.2903/j.efsa.2012.2968>



- EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of vitamin D3 (cholecalciferol) as a feed additive for pigs, piglets, bovines, ovines, calves, equines, chickens for fattening, turkeys, other poultry, fish and other animal species or categories, based on a dossier submitted by Fermenta Biotech Ltd. *EFSA Journal* 2013;11(7):3289, 26 pp. <https://doi.org/10.2903/j.efsa.2013.3289>
- EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of vitamin D3 (cholecalciferol) as a feed additive for all animal species or categories based on a dossier submitted by Lohmann Animal Health GmbH. *EFSA Journal* 2014;12(2):3568, 24 pp. <https://doi.org/10.2903/j.efsa.2014.3568>
- EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2017. Safety of vitamin D3 addition to feedingstuffs for fish. *EFSA Journal* 2017;15(3):4713, 19 pp. <https://doi.org/10.2903/j.efsa.2017.4713>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2012a. Scientific Opinion on the Tolerable Upper Intake Level of calcium. *EFSA Journal* 2012;10(7):2814, 44 pp. <https://doi.org/10.2903/j.efsa.2012.2814>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2012b. Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. *EFSA Journal* 2012;10(7):2813, 45 pp. <https://doi.org/10.2903/j.efsa.2012.2813>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2014. Scientific Opinion on the safety of vitamin D-enriched UV-treated baker's yeast. *EFSA Journal* 2014;12(1):3520, 19 pp. <https://doi.org/10.2903/j.efsa.2014.3520>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2015. Scientific Opinion on Dietary Reference Values for calcium. *EFSA Journal* 2015;13(5):4101, 82 pp. <https://doi.org/10.2903/j.efsa.2015.4101>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2016. Dietary reference values for vitamin D. *EFSA Journal* 2016;14(10):4547, 145 pp. <https://doi.org/10.2903/j.efsa.2016.4547>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2018. Update of the tolerable upper intake level for vitamin D for infants. *EFSA Journal* 2018;16(8):5365, 118 pp. <https://doi.org/10.2903/j.efsa.2018.5365>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2020. Safety of vitamin D2 mushroom powder as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal* 2020;18(1):5948, 23 pp. <https://doi.org/10.2903/j.efsa.2020.5948>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2021a. Safety of calcidiol monohydrate produced by chemical synthesis as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 2021;19(7):6660, 30 pp. <https://doi.org/10.2903/j.efsa.2021.6660>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2021b. Safety of extended uses of UV-treated baker's yeast as a Novel Food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 2021;19(6):6602, 13 pp. <https://doi.org/10.2903/j.efsa.2021.6602>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2021c. Safety of Vitamin D2 mushroom powder (*Agaricus bisporus*) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal* 2021;19(4):6516, 19 pp. <https://doi.org/10.2903/j.efsa.2021.6516>
- EFSA NDA Panel (EFSA Panel on Nutrient, Novel Foods and Food Allergens), 2022. Guidance for establishing and applying tolerable upper intake levels for vitamins and essential minerals. *EFSA Journal* 2022;20(1):200102, 27 pp. <https://doi.org/10.2903/j.efsa.2022.200102>
- EFSA Scientific Committee, 2017a. Guidance on the assessment of the biological relevance of data in scientific assessments. *EFSA Journal* 2017;15(8):4970, 73 pp. <https://doi.org/10.2903/j.efsa.2017.4970>
- EFSA Scientific Committee, 2017b. Guidance on the use of the weight of evidence approach in scientific assessments. *EFSA Journal* 2017;15(8):4971, 69 pp. <https://doi.org/10.2903/j.efsa.2017.4971>
- EFSA Scientific Committee, 2018. Guidance on Uncertainty Analysis in Scientific Assessments. *EFSA Journal* 2018;16(1):5123, 39 pp. <https://doi.org/10.2903/j.efsa.2018.5123>
- EFSA Scientific Committee, 2020. Draft for internal testing Scientific Committee guidance on appraising and integrating evidence from epidemiological studies for use in EFSA's scientific assessments. *EFSA Journal* 2020;18(8):6221, 83 pp. <https://doi.org/10.2903/j.efsa.2020.6221>
- El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A and Vieth R, 2006. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. *The Journal of Clinical Endocrinology and Metabolism*, 91, 405–412. <https://doi.org/10.1210/jc.2005-1436>
- Engelsen O, 2010. The relationship between ultraviolet radiation exposure and vitamin D status. *Nutrients*, 2, 482–495. <https://doi.org/10.3390/nu2050482>
- Engelsen O, Brustad M, Aksnes L and Lund E, 2005. Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. *Photochemistry and Photobiology*, 81, 1287–1290. <https://doi.org/10.1562/2004-11-19-RN-375>
- Enkhmaa D, Tanz L, Ganmaa D, Enkhtur S, Oyun EB, Stuart J, Chen G, Carr A, Seely EW, Fitzmaurice G, Buyandelger Y, Sarantsetseg B, Gantsetseg G and Rich EJ, 2019. Randomized trial of three doses of vitamin D to reduce deficiency in pregnant Mongolian women. *eBioMedicine*, 39, 510–519.



- Ensrud KE, Taylor BC, Paudel ML, Cauley JA, Cawthon PM, Cummings SR, Fink HA, Barrett-Connor E, Zmuda JM, Shikany JM, Orwoll ES and Osteoporotic Fractures in Men Study G, 2009. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. *The Journal of Clinical Endocrinology and Metabolism*, 94, 2773–2780. <https://doi.org/10.1210/jc.2008-2786>
- Espersen R, Ejlsmark-Svensson H, Madsen LR, Nebel C, Danielsen M, Dalsgaard TK and Rejnmark L, 2023. Influence of food matrix delivery system on the bioavailability of vitamin D3: a randomized crossover trial in postmenopausal women. *Nutrition*, 107, 111911. <https://doi.org/10.1016/j.nut.2022.111911>
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2009. Principles and methods for the risk assessment of chemicals in food. *Environmental Health Criteria*, 240.
- Farrar MD, Webb AR, Kift R, Durkin MT, Allan D, Herbert A, Berry JL and Rhodes LE, 2013. Efficacy of a dose range of simulated sunlight exposures in raising vitamin D status in South Asian adults: implications for targeted guidance on sun exposure. *The American Journal of Clinical Nutrition*, 97, 1210–1216. <https://doi.org/10.3945/ajcn.112.052639>
- Flicker L, MacInnis RJ, Stein MS, Scherer SC, Mead KE, Nowson CA, Thomas J, Lowndes C, Hopper JL and Wark JD, 2005. Should older people in residential care receive vitamin D to prevent falls? Results of a randomized trial. *Journal of the American Geriatrics Society*, 53, 1881–1888. <https://doi.org/10.1111/j.1532-5415.2005.00468.x>
- Gaksch M, Jorde R, Grimnes G, Joakimsen R, Schirmer H, Wilsgaard T, Mathiesen EB, Njolstad I, Lochen ML, Marz W, Kleber ME, Tomaschitz A, Grubler M, Eiriksdottir G, Gudmundsson EF, Harris TB, Cotch MF, Aspelund T, Gudnason V, Rutters F, Beulens JW, van 't Riet E, Nijpels G, Dekker JM, Grove-Laugesen D, Rejnmark L, Busch MA, Mensink GB, Scheidt-Nave C, Thamm M, Swart KM, Brouwer IA, Lips P, van Schoor NM, Sempos CT, Durazo-Arvizu RA, Skrabakova Z, Dowling KG, Cashman KD, Kiely M and Pilz S, 2017. Vitamin D and mortality: individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium. *PLoS One*, 12, e0170791. <https://doi.org/10.1371/journal.pone.0170791>
- Gallagher JC, Sai A, Templin T and Smith L, 2012. Dose response to vitamin D supplementation in postmenopausal women a randomized trial. *Annals of Internal Medicine*, 156, 425–U476. <https://doi.org/10.7326/0003-4819-156-6-201203200-00005>
- Gallagher JC, Peacock M, Yalamanchili V and Smith LM, 2013. Effects of vitamin D supplementation in older African American women. *The Journal of Clinical Endocrinology and Metabolism*, 98, 1137–1146.
- Gallagher JC, Smith LM and Yalamanchili V, 2014a. Incidence of hypercalciuria and hypercalcemia during vitamin D and calcium supplementation in older women. *Menopause*, 21, 1173–1180.
- Gallagher JC, Jindal PS and Smith LM, 2014b. Vitamin D supplementation in young White and African American women. *Journal of Bone and Mineral Research*, 29, 173–181. <https://doi.org/10.1002/jbmr.2010>
- Gallo S, Phan A, Vanstone CA, Rodd C and Weiler HA, 2013. The change in plasma 25-hydroxyvitamin D did not differ between breast-fed infants that received a daily supplement of ergocalciferol or cholecalciferol for 3 months. *The Journal of Nutrition*, 143, 148–153. <https://doi.org/10.3945/jn.112.167858>
- Gangloff A, Bergeron J, Pelletier-Beaumont E, Nazare JA, Smith J, Borel AL, Lemieux I, Tremblay A, Poirier P, Almeras N and Despres JP, 2015. Effect of adipose tissue volume loss on circulating 25-hydroxyvitamin D levels: results from a 1-year lifestyle intervention in viscerally obese men. *International Journal of Obesity*, 39, 1638–1643. <https://doi.org/10.1038/ijo.2015.118>
- Garland CF, Kim JJ, Mohr SB, Gorham ED, Grant WB, Giovannucci EL, Baggerly L, Hofflich H, Ramsdell JW, Zeng K and Heaney RP, 2014. Meta-analysis of all-cause mortality according to serum 25-hydroxyvitamin D. *American Journal of Public Health*, 104, e43–e50. <https://doi.org/10.2105/AJPH.2014.302034>
- Ginde AA, Scragg R, Schwartz RS and Camargo CA, 2009. Prospective study of serum 25-hydroxyvitamin D level, cardiovascular disease mortality, and all-cause mortality in older US adults. *Journal of the American Geriatrics Society*, 57, 1595–1603. <https://doi.org/10.1111/j.1532-5415.2009.02359.x>
- Gonnelli S, Tomai Pitinca MD, Camarri S, Lucani B, Franci B, Nuti R and Caffarelli C, 2021. Pharmacokinetic profile and effect on bone markers and muscle strength of two daily dosage regimens of calcifediol in osteopenic/osteoporotic postmenopausal women. *Aging Clinical and Experimental Research*, 33, 2539–2547. <https://doi.org/10.1007/s40520-020-01779-7>
- Gordon CM, Williams AL, Feldman HA, May J, Sinclair L, Vasquez A and Cox JE, 2008. Treatment of hypovitaminosis D in infants and toddlers. *The Journal of Clinical Endocrinology and Metabolism*, 93, 2716–2721. <https://doi.org/10.1210/jc.2007-2790>
- Graeff-Armas LA, Bendik I, Kunz I, Schoop R, Hull S and Beck M, 2020. Supplemental 25-hydroxycholecalciferol is more effective than cholecalciferol in raising serum 25-hydroxyvitamin D concentrations in older adults. *The Journal of Nutrition*, 150, 73–81. <https://doi.org/10.1093/jn/nxz209>
- Grant WB, 2015. Letter to the Editor: The J-shaped 25-hydroxyvitamin D concentration-cardiovascular disease mortality relation is very likely due to starting vitamin D supplementation late in life. *The Journal of Clinical Endocrinology and Metabolism*, 100, L49–L50. <https://doi.org/10.1210/jc.2015-1554>
- Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, Anderson FH, Cooper C, Francis RM, Donaldson C, et al., 2005. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet (London, England)*, 365, 1621–1628. [https://doi.org/10.1016/s0140-6736\(05\)63013-9](https://doi.org/10.1016/s0140-6736(05)63013-9)

- Grant WB, Karras SN, Bischoff-Ferrari HA, Annweiler C, Boucher BJ, Juzeniene A, Garland CF and Holick MF, 2016. Do studies reporting 'U'-shaped serum 25-hydroxyvitamin D-health outcome relationships reflect adverse effects? *Dermatoendocrinol*, 8, e1187349. <https://doi.org/10.1080/19381980.2016.1187349>
- Grimnes G, Joakimsen R, Figenschau Y, Torjesen PA, Almas B and Jorde R, 2012. The effect of high-dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass—a randomized controlled 1-year trial. *Osteoporosis International*, 23, 201–211. <https://doi.org/10.1007/s00198-011-1752-5>
- Gropper S, Smith J and Groff J, 2009. *Advanced Nutrition & Human Metabolism*. Wadsworth Cengage Learning, Belmont, MA, USA. 587 p.
- Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, Jani BD, Welsh P, Mair FS, Gray SR, O'Donnell CA, Gill JM, Sattar N and Pell JP, 2020. Vitamin D concentrations and COVID-19 infection in UK Biobank. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 14, 561–565. <https://doi.org/10.1016/j.dsx.2020.04.050>
- Hauksson HH, Hrafnkelsson H, Magnusson KT, Johannsson E and Sigurdsson EL, 2016. Vitamin D status of Icelandic children and its influence on bone accrual. *Journal of Bone and Mineral Metabolism*, 34, 580–586. <https://doi.org/10.1007/s00774-015-0704-0>
- Heaney RP, Davies KM, Chen TC, Holick MF and Barger-Lux MJ, 2003. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *American Journal of Clinical Nutrition*, 77, 204–210. <https://doi.org/10.1093/ajcn/77.1.204>
- Heaney RP, Horst RL, Cullen DM and Armas LA, 2009. Vitamin D3 distribution and status in the body. *Journal of the American College of Nutrition*, 28, 252–256. <https://doi.org/10.1080/07315724.2009.10719779>
- Herrick KA, Storaardt RJ, Afful J, Pfeiffer CM, Schleicher RL, Gahche JJ and Potischman N, 2019. Vitamin D status in the United States, 2011–2014. *The American Journal of Clinical Nutrition*, 110, 150–157. <https://doi.org/10.1093/ajcn/nqz037>
- Heuer T, Walter C, Krems C and Hoffmann I (Max Rubner-Institut), 2012. Nährstoffzufuhr über Supplemente – Ergebnisse der Nationalen Verzehrsstudie II. 86–97 pp.
- Himbert C, Ose J, Delphan M and Ulrich CM, 2017. A systematic review of the interrelation between diet- and surgery-induced weight loss and vitamin D status. *Nutrition Research*, 38, 13–26. <https://doi.org/10.1016/j.nutres.2016.12.004>
- Himmelstein S, Clemens TL, Rubin A and Lindsay R, 1990. Vitamin D supplementation in elderly nursing home residents increases 25(OH)D but not 1,25(OH)2D. *American Journal of Clinical Nutrition*, 52, 701–706.
- Hin H, Tomson J, Newman C, Kurien R, Lay M, Cox J, Sayer J, Hill M, Emberson J, Armitage J and Clarke R, 2016. Optimum dose of vitamin D for disease prevention in older people: BEST-D trial of vitamin D in primary care. *Osteoporosis International*, 28, 841–851.
- Hindborg H (DTU Food National Institute), 2015. Unpublished. Dietary supplements may contribute to an adequate micronutrient intake in Danish adults but increase the risk of overdose in children.
- Holick MF, 1988. Skin: site of the synthesis of vitamin D and a target tissue for the active form, 1,25-dihydroxyvitamin D3. *Annals of the New York Academy of Sciences*, 548, 14–26. <https://doi.org/10.1111/j.1749-6632.1988.tb18789.x>
- Hollis BW and Wagner CL, 2004. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *American Journal of Clinical Nutrition*, 80, 1752S–1758S. <https://doi.org/10.1093/ajcn/80.6.1752S>
- Hollis BW, Johnson D, Hulsey TC, Ebeling M and Wagner CL, 2011. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *Journal of Bone and Mineral Research*, 26, 2341–2357. <https://doi.org/10.1002/jbmr.463>
- Holmberg I, Berlin T, Ewerth S and Björkhem I, 1986. 25-Hydroxylase activity in subcellular fractions from human liver. Evidence for different rates of mitochondrial hydroxylation of vitamin D2 and D3. *Scandinavian Journal of Clinical and Laboratory Investigation*, 46, 785–790. <https://doi.org/10.3109/00365518609084051>
- Houghton LA and Vieth R, 2006. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *The American Journal of Clinical Nutrition*, 84, 694–697. <https://doi.org/10.1093/ajcn/84.4.694>
- Hribar M, Hristov H, Lavriša Ž, Koroušić Seljak B, Gregorič M, Blaznik U, Žmitek K and Pravst I, 2021. Vitamin D intake in Slovenian adolescents, adults, and the elderly population. *Nutrients*, 13. <https://doi.org/10.3390/nu13103528>
- Hu HL, Zhang JQ, Lu YC, Zhang ZB, Qin BL, Gao HB, Wang YF, Zhu JN, Wang Q, Zhu YP, Xun Y and Wang SG, 2017. Association between circulating vitamin D level and urolithiasis: a systematic review and meta-analysis. *Nutrients*, 9. <https://doi.org/10.3390/nu9030301>
- Hutchinson MS, Grimnes G, Joakimsen RM, Figenschau Y and Jorde R, 2010. Low serum 25-hydroxyvitamin D levels are associated with increased all-cause mortality risk in a general population: the Tromsø study. *European Journal of Endocrinology*, 162, 935–942. <https://doi.org/10.1530/EJE-09-1041>
- Hyponen E and Boucher BJ, 2018. Adiposity, vitamin D requirements, and clinical implications for obesity-related metabolic abnormalities. *Nutrition Reviews*, 76, 678–692. <https://doi.org/10.1093/nutrit/nuy034>
- IOM, 1997. *The National Academies Collection: Reports funded by National Institutes of Health. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academy of Sciences, Washington (DC), National Academies Press (US) Copyright © 1997.

- IOM, 2011. The National Academies Collection: Reports funded by National Institutes of Health. In: AC Ross, CL Taylor, AL Yaktine and HB Del Valle (eds). Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press (US) Copyright © 2011, National Academy of Sciences, Washington (DC).
- Itkonen ST, Skaffari E, Saaristo P, Saarnio EM, Erkkola M, Jakobsen J, Cashman KD and Lamberg-Allardt C, 2016. Effects of vitamin D2-fortified bread v. supplementation with vitamin D2 or D3 on serum 25-hydroxyvitamin D metabolites: an 8-week randomised-controlled trial in young adult Finnish women. *The British Journal of Nutrition*, 115, 1232–1239. <https://doi.org/10.1017/s0007114516000192>
- Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, Bassford T, Beresford SA, Black HR, Blanchette P, Bonds DE, Brunner RL, Brzyski RG, Caan B, Cauley JA, Chlebowski RT, Cummings SR, Granek I, Hays J, Heiss G, Hendrix SL, Howard BV, Hsia J, Hubbell FA, Johnson KC, Judd H, Kotchen JM, Kuller LH, Langer RD, Lasser NL, Limacher MC, Ludlam S, Manson JE, Margolis KL, McGowan J, Ockene JK, O'Sullivan MJ, Phillips L, Prentice RL, Sarto GE, Stefanick ML, Van Horn L, Wactawski-Wende J, Whitlock E, Anderson GL, Assaf AR, Barad D and Women's Health Initiative I, 2006. Calcium plus vitamin D supplementation and the risk of fractures. *The New England Journal of Medicine*, 354, 669–683. <https://doi.org/10.1056/NEJMoa055218>
- Jakobsen J and Saxholt E, 2009. Vitamin D metabolites in bovine milk and butter. *Journal of Food Composition and Analysis*, 22, 472–478. <https://doi.org/10.1016/j.jfca.2009.01.010>
- Jakobsen J, Andersen EAW, Christensen T, Andersen R and Bugel S, 2017. Vitamin D vitamers affect vitamin D status differently in young healthy males. *Nutrients*, 10, 23. <https://doi.org/10.3390/nu10010012>
- Janousek J, Pilarova V, Macakova K, Nomura A, Veiga-Matos J, Silva DDD, Remiao F, Saso L, Mala-Ladova K, Maly J, Novakova L and Mladenka P, 2022. Vitamin D: sources, physiological role, biokinetics, deficiency, therapeutic use, toxicity, and overview of analytical methods for detection of vitamin D and its metabolites. *Critical Reviews in Clinical Laboratory Sciences*, 59, 517–554. <https://doi.org/10.1080/10408363.2022.2070595>
- Jia X, Aucott LS and McNeill G, 2007. Nutritional status and subsequent all-cause mortality in men and women aged 75 years or over living in the community. *The British Journal of Nutrition*, 98, 593–599. <https://doi.org/10.1017/s0007114507725163>
- Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, Ingelsson E, Karasik D, Pilz S, Berry D, Kestenbaum B, Zheng J, Luan J, Sofianopoulou E, Streeten EA, Albanes D, Lutsey PL, Yao L, Tang W, Econs MJ, Wallaschofski H, Völzke H, Zhou A, Power C, McCarthy MI, Michos ED, Boerwinkle E, Weinstein SJ, Freedman ND, Huang WY, Van Schoor NM, van der Velde N, Groot L, Enneman A, Cupples LA, Booth SL, Vasani RS, Liu CT, Zhou Y, Ripatti S, Ohlsson C, Vandenput L, Lorentzon M, Eriksson JG, Shea MK, Houston DK, Kritchevsky SB, Liu Y, Lohman KK, Ferrucci L, Peacock M, Gieger C, Beekman M, Slagboom E, Deelen J, Heemst DV, Kleber ME, März W, de Boer IH, Wood AC, Rotter JI, Rich SS, Robinson-Cohen C, den Heijer M, Jarvelin MR, Cavadino A, Joshi PK, Wilson JF, Hayward C, Lind L, Michaëlsson K, Trompet S, Zillikens MC, Uitterlinden AG, Rivadeneira F, Broer L, Zgaga L, Campbell H, Theodoratou E, Farrington SM, Timofeeva M, Dunlop MG, Valdes AM, Tikkanen E, Lehtimäki T, Lytykäinen LP, Kähönen M, Raitakari OT, Mikkilä V, Ikram MA, Sattar N, Jukema JW, Wareham NJ, Langenberg C, Forouhi NG, Gundersen TE, Khaw KT, Butterworth AS, Danesh J, Spector T, Wang TJ, Hyppönen E, Kraft P and Kiel DP, 2018. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nature Communications*, 9, 260. <https://doi.org/10.1038/s41467-017-02662-2>
- Johansson H, Odén A, Kanis J, McCloskey E, Lorentzon M, Ljunggren Ö, Karlsson MK, Thorsby PM, Tivesten Å, Barrett-Connor E, Ohlsson C and Mellström D, 2012. Low serum vitamin D is associated with increased mortality in elderly men: MrOS Sweden. *Osteoporosis International*, 23, 991–999. <https://doi.org/10.1007/s00198-011-1809-5>
- Johnson KC, Pittas AG, Margolis KL, Peters AL, Phillips LS, Vickery EM, Nelson J, Sheehan PR, Reboussin D, Malozowski S and Chatterjee R, 2022. Safety and tolerability of high-dose daily vitamin D3 supplementation in the vitamin D and type 2 diabetes (D2d) study—a randomized trial in persons with prediabetes. *European Journal of Clinical Nutrition*, 76, 1117–1124. <https://doi.org/10.1038/s41430-022-01068-8>
- Jones G, 2013. Extrarenal Vitamin D Activation and Interactions Between Vitamin D<sub>2</sub>, Vitamin D<sub>3</sub>, and Vitamin D Analogs. *Annual Review of Nutrition*, 33, 23–44. <https://doi.org/10.1146/annurev-nutr-071812-161203>
- Jones G, 2014. Vitamin D. In: ACCB Ross, RJ Cousins, KL Tucker and TR Ziegler (eds). *Modern nutrition in health and disease*. Wolters Kluwer Health/Lippincott Williams & Wilkins, Baltimore, Philadelphia, PA, USA. pp. 278–292.
- Jones G, Prosser DE and Kaufmann M, 2012. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Archives of Biochemistry and Biophysics*, 523, 9–18. <https://doi.org/10.1016/j.abb.2011.11.003>
- Jones KS, Assar S, Harnpanich D, Bouillon R, Lambrechts D, Prentice A and Schoenmakers I, 2014. 25(OH)D<sub>2</sub> half-life is shorter than 25(OH)D<sub>3</sub> half-life and is influenced by DBP concentration and genotype. *The Journal of Clinical Endocrinology and Metabolism*, 99, 3373–3381. <https://doi.org/10.1210/jc.2014-1714>
- Jones G, Kottler ML and Schlingmann KP, 2017. Genetic diseases of vitamin D metabolizing enzymes. *Endocrinology and Metabolism Clinics of North America*, 46, 1095–1117. <https://doi.org/10.1016/j.ecl.2017.07.011>
- Jorde R and Grimnes G, 2020. Serum PTH is not a good marker for defining a threshold for vitamin D deficiency. *Endocrine Connections*, 9, 396–404. <https://doi.org/10.1530/ec-20-0067>

- Jorde R, Sneve M, Figenschau Y, Svartberg J and Waterloo K, 2008. Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. *Journal of Internal Medicine*, 264, 599–609.
- Jorde R, Sneve M, Torjesen PA, Figenschau Y, Hansen JB and Grimnes G, 2010. No significant effect on bone mineral density by high doses of vitamin D3 given to overweight subjects for one year. *Nutrition Journal*, 9, 1. <https://doi.org/10.1186/1475-2891-9-1>
- Jorde R, Sollid ST, Svartberg J, Joakimsen RM, Grimnes G and Hutchinson MYS, 2016. Prevention of urinary tract infections with vitamin D supplementation 20,000 IU per week for five years. Results from an RCT including 511 subjects. *Infectious Diseases*, 48, 823–828. <https://doi.org/10.1080/23744235.2016.1201853>
- Jorde R, Stunes AK, Kubiak J, Joakimsen R, Grimnes G, Thorsby PM and Syversen U, 2019. Effects of vitamin D supplementation on bone turnover markers and other bone-related substances in subjects with vitamin D deficiency. *Bone*, 124, 7–13.
- JPT H, Thomas J, Chandler J, Cumpston M, Li T, Page MJ and Welch VA, 2022. *Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022)*. Cochrane, 2022. Available online: [www.training.cochrane.org/handbook](http://www.training.cochrane.org/handbook)
- Kanis JA, 2008. Assessment of osteoporosis at the primary health-care level. Technical Report. Available online: <http://www.shef.ac.uk/FRAX>
- Kehoe L and Walton J (Munster Technological University), 2022. Report on the intake of micronutrients (Vitamin A,  $\beta$ -carotene, retinol, vitamin D, vitamin E, vitamin B6, folic acid, iron and manganese) from food supplements in the Irish population.
- Keum N, Lee DH, Greenwood DC, Manson JE and Giovannucci E, 2019. Vitamin D supplementation and total cancer incidence and mortality: a meta-analysis of randomized controlled trials. *Annals of Oncology*, 30, 733–743. <https://doi.org/10.1093/annonc/mdz059>
- Khan M, Jose A and Sharma S, 2022. Physiology, Parathyroid Hormone. [Updated 2022 Oct 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK499940/>.
- Kristensen HL, Rosenqvist E and Jakobsen J, 2012. Increase of vitamin D(2) by UV-B exposure during the growth phase of white button mushroom (*Agaricus bisporus*). *Food and Nutrition Research*, 56, 7114. <https://doi.org/10.3402/fnr.v56i0.7114>
- Lamberg-Allardt C, Adebayo F, Peltonen H, Christiansen JJ and Olsen T, 2023. Preparatory work for the update of the tolerable upper intake levels for vitamin D. EFSA supporting publication 2023;EN-7941, 257 pp. <https://doi.org/10.2903/sp.efsa.2023.EN-7941>. Available online: <https://www.efsa.europa.eu/en/supporting/pub/en-7941>
- Larsen AU, Grimnes G and Jorde R, 2017. The effect of high-dose vitamin D3 supplementation on bone mineral density in subjects with prediabetes. *Osteoporosis International*, 29, 171–180. <https://doi.org/10.1007/s00198-017-4222-x>
- LeBoff MS, Murata EM, Cook NR, Cawthon P, Chou SH, Kotler G, Bubes V, Buring JE and Manson JE, 2020a. VITamin D and Omega-3 Trial (VITAL): effects of vitamin D supplements on risk of falls in the US population. *Journal of Clinical Endocrinology and Metabolism*, 105, 2929–2938. <https://doi.org/10.1210/clinem/dgaa311>
- LeBoff MS, Chou SH, Murata EM, Donlon CM, Cook NR, Mora S, Lee IM, Kotler G, Bubes V and Buring JE, 2020b. Effects of supplemental vitamin D on bone health outcomes in women and men in the VITamin D and omega-3 trial (VITAL). *Journal of Bone and Mineral Research*, 35, 883–893. <https://doi.org/10.1002/jbmr.3958>
- Lehmann U, Hirche F, Stangl GI, Hinz K, Westphal S and Dierkes J, 2013. Bioavailability of vitamin D(2) and D(3) in healthy volunteers, a randomized placebo-controlled trial. *The Journal of Clinical Endocrinology and Metabolism*, 98, 4339–4345. <https://doi.org/10.1210/jc.2012-4287>
- Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KM, Leino AE and Viikari JS, 2002. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. *American Journal of Clinical Nutrition*, 76, 1446–1453.
- Letavernier E and Daudon M, 2018. Vitamin D, Hypercalciuria and Kidney Stones. *Nutrients*, 10. <https://doi.org/10.3390/nu10030366>
- Lewis RD, Laing EM, Hill GKM, Hall DB, McCabe GP, Hausman DB, Martin BR, Warden SJ, Peacock M and Weaver CM, 2013. A randomized trial of vitamin D supplementation in children: dose-response effects on vitamin D metabolites and calcium absorption. *Journal of Clinical Endocrinology and Metabolism*, 98, 4816–4825. <https://doi.org/10.1210/jc.2013-2728>
- Lindén V, 1974. Vitamin D and myocardial infarction. *British Medical Journal*, 3, 647–650. <https://doi.org/10.1136/bmj.3.5932.647>
- Lips P, 2007. Relative value of 25(OH)D and 1,25(OH)2D measurements. *Journal of Bone and Mineral Research*, 22, 1668–1671. <https://doi.org/10.1359/jbmr.070716>
- Liu D, Meng X, Tian Q, Cao W, Fan X, Wu L, Song M, Meng Q, Wang W and Wang Y, 2022. Vitamin D and multiple health outcomes: an umbrella review of observational studies, randomized controlled trials, and mendelian randomization studies. *Advances in Nutrition*, 13, 1044–1062. <https://doi.org/10.1093/advances/nmab142>
- Lo CW, Paris PW, Clemens TL, Nolan J and Holick MF, 1985. Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes. *The American Journal of Clinical Nutrition*, 42, 644–649. <https://doi.org/10.1093/ajcn/42.4.644>



- Maalouf J, Nabulsi M, Vieth R, Kimball S, El-Rassi R, Mahfoud Z and El-Hajj Fuleihan G, 2008. Short- and long-term safety of weekly high-dose vitamin D-3 supplementation in school children. *The Journal of Clinical Endocrinology and Metabolism*, 93, 2693–2701. <https://doi.org/10.1210/jc.2007-2530>
- Macdonald HM, Wood AD, Aucott LS, Black AJ, Fraser WD, Mavroeidi A, Reid DM, Secombes KR, Simpson WG and Thies F, 2013. Hip bone loss is attenuated with 1000 IU but not 400 IU daily vitamin D3: a 1-year double-blind RCT in postmenopausal women. *Journal of Bone and Mineral Research*, 28, 2202–2213. <https://doi.org/10.1002/jbmr.1959>
- Malihi Z, Wu Z, Stewart AW, Lawes CM and Scragg R, 2016. Hypercalcemia, hypercalciuria, and kidney stones in long-term studies of vitamin D supplementation: a systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, 104, 1039–1051. <https://doi.org/10.3945/ajcn.116.134981>
- Malihi Z, Wu Z, Lawes CMM and Scragg R, 2019. Adverse events from large dose vitamin D supplementation taken for one year or longer. *The Journal of Steroid Biochemistry and Molecular Biology*, 188, 29–37. <https://doi.org/10.1016/j.jsbmb.2018.12.002>
- Malmberg P, Karlsson T, Svensson H, Lönn M, Carlsson NG, Sandberg AS, Jennische E, Osmancevic A and Holmäng A, 2014. A new approach to measuring vitamin D in human adipose tissue using time-of-flight secondary ion mass spectrometry: a pilot study. *Journal of Photochemistry and Photobiology. B*, 138, 295–301. <https://doi.org/10.1016/j.jphotobiol.2014.06.008>
- Manson JE, Bassuk SS, Buring JE and Group VR, 2020. Principal results of the VITamin D and Omega-3 Trial (VITAL) and updated meta-analyses of relevant vitamin D trials. *The Journal of Steroid Biochemistry and Molecular Biology*, 198, 105522. <https://doi.org/10.1016/j.jsbmb.2019.105522>
- Mastaglia SR, Mautalen CA, Parisi MS and Oliveri B, 2006. Vitamin D2 dose required to rapidly increase 25OHD levels in osteoporotic women. *European Journal of Clinical Nutrition*, 60, 681–687.
- Melamed ML, Muntner P, Michos ED, Uribarri J, Weber C, Sharma J and Raggi P, 2008. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease - results from NHANES 2001 to 2004. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 28, 1179–1185. <https://doi.org/10.1161/atvbaha.108.165886>
- Mensink GB, Haftenberger M, Lage-Barbosa C, Brettschneider AK, Lehmann F, Frank M, Heide K, Moosburger R, Patelakis E and Perlit H, 2021. EsKiMo II - Die Ernährungsstudie als KiGGS-Modul. <https://doi.org/10.25646/7028.2>
- Michaelsson K, Baron JA, Snellman G, Gedeberg R, Byberg L, Sundstrom J, Berglund L, Arnlov J, Hellman P, Blomhoff R, Wolk A, Garmo H, Holmberg L and Melhus H, 2010. Plasma vitamin D and mortality in older men: a community-based prospective cohort study. *The American Journal of Clinical Nutrition*, 92, 841–848. <https://doi.org/10.3945/ajcn.2010.29749>
- Minisola S, Cianferotti L, Biondi P, Cipriani C, Fossi C, Franceschelli F, Giusti F, Leoncini G, Pepe J, Bischoff-Ferrari HA and Brandi ML, 2017. Correction of vitamin D status by calcidiol: pharmacokinetic profile, safety, and biochemical effects on bone and mineral metabolism of daily and weekly dosage regimens. *Osteoporosis International*, 28, 3239–3249.
- Mirhosseini N, Rainsbury J and Kimball SM, 2018. Vitamin D supplementation, serum 25(OH)D concentrations and cardiovascular disease risk factors: a systematic review and meta-analysis. *Frontiers of Cardiovascular Medicine*, 5, 87. <https://doi.org/10.3389/fcvm.2018.00087>
- Mitchell BL, Zhu G, Medland SE, Renteria ME, Eyles DW, Grasby KL, McGrath JJ and Martin NG, 2019. Half the genetic variance in vitamin D concentration is shared with skin colour and sun exposure genes. *Behavior Genetics*, 49, 386–398. <https://doi.org/10.1007/s10519-019-09954-x>
- Moyersoen I, Devleeschauwer B, Dekkers A, De Ridder K, Tafforeau J, van Camp J, van Oyen H and Lachat C, 2017. Intake of fat-soluble vitamins in the Belgian population: adequacy and contribution of foods, fortified foods and supplements. *Nutrients*, 9. <https://doi.org/10.3390/nu9080860>
- Natri AM, Salo P, Vikstedt T, Palssa A, Huttunen M, Kärkkäinen MU, Salovaara H, Piironen V, Jakobsen J and Lamberg-Allardt CJ, 2006. Bread fortified with cholecalciferol increases the serum 25-hydroxyvitamin D concentration in women as effectively as a cholecalciferol supplement. *The Journal of Nutrition*, 136, 123–127. <https://doi.org/10.1093/jn/136.1.123>
- Navarro-Valverde C, Sosa-Henriquez M, Alhambra-Exposito MR and Quesada-Gomez JM, 2016. Vitamin D3 and calcidiol are not equipotent. *The Journal of Steroid Biochemistry and Molecular Biology*, 164, 205–208. <https://doi.org/10.1016/j.jsbmb.2016.01.014>
- Neale RE, Baxter C, Romero BD, McLeod DSA, English DR, Armstrong BK, Ebeling PR, Hartel G, Kimlin MG, O'Connell R, van der Pols JC, Venn AJ, Webb PM, Whiteman DC and Waterhouse M, 2022. The D-Health Trial: a randomised controlled trial of the effect of vitamin D on mortality. *The Lancet Diabetes and Endocrinology*, 10, 120–128. [https://doi.org/10.1016/s2213-8587\(21\)00345-4](https://doi.org/10.1016/s2213-8587(21)00345-4)
- NHMRC (National Health and Medical Research Council, New Zealand. Ministry of H, Australia. Department of H and Ageing), 2006. Nutrient reference values for Australia and New Zealand : including recommended dietary intakes. [Canberra, A.C.T, National Health and Medical Research Council].
- Nikooyeh B, Zahedirad M, Kalayi A, Shariatzadeh N, Hollis BW and Neyestani TR, 2022. Improvement of vitamin D status through consumption of either fortified food products or supplement pills increased hemoglobin concentration in adult subjects: analysis of pooled data from two randomized clinical trials. *Nutrition and Health*. <https://doi.org/10.1177/02601060221085351>

- Nissen J, Vogel U, Ravn-Haren G, Andersen EW, Madsen KH, Nexø BA, Andersen R, Mejborn H, Bjerrum PJ, Rasmussen LB and Wulf HC, 2015. Common variants in CYP2R1 and GC genes are both determinants of serum 25-hydroxyvitamin D concentrations after UVB irradiation and after consumption of vitamin D-fortified bread and milk during winter in Denmark. *The American Journal of Clinical Nutrition*, 101, 218–227. <https://doi.org/10.3945/ajcn.114.092148>
- Nygaard B, Frandsen NE, Brandi L, Rasmussen K, Oestergaard OV, Oedum L, Hoeck HC and Hansen D, 2014. Effects of high doses of cholecalciferol in normal subjects: a randomized double-blinded, Placebo-Controlled Trial. *PLoS One*, 9, e102965. <https://doi.org/10.1371/journal.pone.0102965>
- OHAT-NTP (Office of Health Assessment and Translation-Division of the National Toxicology Program), 2015. OHAT risk of bias rating tool for human and animal studies. 37 pp. Available online: [https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool\\_508.pdf](https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf)
- Okoye C, Calsolaro V, Niccolai F, Calabrese AM, Franchi R, Rogani S, Coppini G, Morelli V, Caraccio N and Monzani F, 2022. A randomized, open-label study to assess efficacy of weekly assumption of cholecalciferol versus calcifediol in older patients with hypovitaminosis D. *Geriatrics*, 7, 7. <https://doi.org/10.3390/geriatrics7010013>
- Peacock M, 2010. Calcium metabolism in health and disease. *Clinical Journal of the American Society of Nephrology*, 5(Suppl 1), S23–S30. <https://doi.org/10.2215/CJN.05910809>
- Peacock M, Liu G, Carey M, McClintock R, Ambrosius W, Hui S and Johnston CC, 2000. Effect of calcium or 25OH vitamin D3 dietary supplementation on bone loss at the hip in men and women over the age of 60. *Journal of Clinical Endocrinology and Metabolism*, 85, 3011–3019. <https://doi.org/10.1210/jcem.85.9.6836>
- Pei YY, Zhang Y, Peng XC, Liu ZR, Xu P and Fang F, 2022. Association of vitamin D supplementation with cardiovascular events: a systematic review and meta-analysis. *Nutrients*, 14. <https://doi.org/10.3390/nu14153158>
- Perlitz H, Mensink GBM, Lage Barbosa C, Richter A, Brettschneider AK, Lehmann F, Patelakis E, Frank M, Heide K and Haftenberger M, 2019. Use of vitamin and mineral supplements among adolescents living in Germany-Results from ESKiMo II. *Nutrients*, 11. <https://doi.org/10.3390/nu11061208>
- Pike JW and Christakos S, 2017. Biology and mechanisms of action of the vitamin D hormone. *Endocrinology and Metabolism Clinics of North America*, 46, 815–843. <https://doi.org/10.1016/j.jec.2017.07.001>
- Ponda MP, Dowd K, Finkelstein D, Holt PR and Breslow JL, 2012. The short-term effects of vitamin D repletion on cholesterol: a randomized, placebo-controlled trial. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 32, 2510–2515.
- Prince RL, Austin N, Devine A, Dick IM, Bruce D and Zhu K, 2008. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. *Archives of Internal Medicine*, 168, 103–108. <https://doi.org/10.1001/archinternmed.2007.31>
- Pronicka E, Ciara E, Halat P, Janiec A, Wójcik M, Rowińska E, Rokicki D, Płudowski P, Wojciechowska E, Wierzbicka A, Książek JB, Jacoszek A, Konrad M, Schlingmann KP and Litwin M, 2017. Biallelic mutations in CYP24A1 or SLC34A1 as a cause of infantile idiopathic hypercalcemia (IIH) with vitamin D hypersensitivity: molecular study of 11 historical IIH cases. *Journal of Applied Genetics*, 58, 349–353. <https://doi.org/10.1007/s13353-017-0397-2>
- Quesada-Gomez JM and Bouillon R, 2018. Is calcifediol better than cholecalciferol for vitamin D supplementation? *Osteoporosis International*, 29, 1697–1711. <https://doi.org/10.1007/s00198-018-4520-y>
- Rafii DC, Ali F, Farag A, Iyer B, Otterbeck PE, Chaudhari R, Potter N, Stefanov DG and Guber HA, 2019. A prospective study of commonly utilized regimens of Vitamin D replacement and maintenance therapy in adults. *Endocrine Practice*, 25, 6–15.
- Rahme M, Sharara SL, Baddoura R, Habib RH, Halaby G, Arabi A, Singh RJ, Kassem M, Mahfoud Z, Hoteit M, Daher RT, Bassil D, El FK and El-Hajj FG, 2017. Impact of calcium and two doses of vitamin D on bone metabolism in the elderly: a randomized controlled trial. *Journal of Bone and Mineral Research*, 32, 1486–1495. <https://doi.org/10.1002/jbmr.3122>
- Rajakumar K, Moore CG, Yabes J, Olabopo F, Haralam MA, Comer D, Bogusz J, Nucci A, Sereika S and Dunbar-Jacob J, 2015. Effect of vitamin D3 supplementation in black and in white children: a randomized, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism*, 100, 3183–3192. <https://doi.org/10.1210/jc.2015-1643>
- Rajakumar K, Moore CG, Khalid AT, Vallejo AN, Virji MA, Holick MF, Greenspan SL, Arslanian S and Reis SE, 2020. Effect of vitamin D3 supplementation on vascular and metabolic health of vitamin D-deficient overweight and obese children: a randomized clinical trial. *American Journal of Clinical Nutrition*, 111, 757–768.
- Reboul E, 2015. Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food and Function*, 6, 356–362. <https://doi.org/10.1039/c4fo00579a>
- Rejnmark L, Bislev LS, Cashman KD, Eiriksdottir G, Gaksch M, Grubler M, Grimnes G, Gudnason V, Lips P, Pilz S, van Schoor NM, Kiely M and Jorde R, 2017. Non-skeletal health effects of vitamin D supplementation: a systematic review on findings from meta-analyses summarizing trial data. *PLoS One*, 12, e0180512. <https://doi.org/10.1371/journal.pone.0180512>
- Revez JA, Lin T, Qiao Z, Xue A, Holtz Y, Zhu Z, Zeng J, Wang H, Sidorenko J, Kemper KE, Vinkhuyzen AAE, Frater J, Eyles D, Burne THJ, Mitchell B, Martin NG, Zhu G, Visscher PM, Yang J, Wray NR and McGrath JJ, 2020. Genome-wide association study identifies 143 loci associated with 25 hydroxyvitamin D concentration. *Nature Communications*, 11, 1647. <https://doi.org/10.1038/s41467-020-15421-7>

- Rizzoli R, 2021. Vitamin D supplementation: upper limit for safety revisited? *Aging Clinical and Experimental Research*, 33, 19–24. <https://doi.org/10.1007/s40520-020-01678-x>
- Roe MA, Bell S, Oseredczuk M, Christensen T, Westenbrink S, Pakkala H, Presser K and Finglas PM, 2013. Updated food composition database for nutrient intake. EFSA Supporting Publications, 10, 355E. <https://doi.org/10.2903/sp.efsa.2013.EN-355>
- Rorie A, Goldner WS, Lyden E and Poole JA, 2014. Beneficial role for supplemental vitamin D3 treatment in chronic urticaria: a randomized study. *Annals of Allergy, Asthma and Immunology*, 112, 376–382. <https://doi.org/10.1016/j.anai.2014.01.010>
- Roth DE, Morris SK, Zlotkin S, Gernand AD, Ahmed T, Shanta SS, Papp E, Korsiak J, Shi J, Islam MM, Jahan I, Keya FK, Willan AR, Weksberg R, Mohsin M, Rahman QS, Shah PS, Murphy KE, Stimec J, Pell LG, Qamar H and al Mahmud A, 2018. Vitamin D supplementation in pregnancy and lactation and infant growth. *New England Journal of Medicine*, 379, 535–546. <https://doi.org/10.1056/NEJMoa1800927>
- Ruggiero C, Baroni M, Bini V, Brozzetti A, Parretti L, Zengarini E, Lapenna M, Antinolfi P, Falorni A, Mecocci P and Boccardi V, 2019. Effects of weekly supplementation of cholecalciferol and calcifediol among the oldest-old people: findings from a randomized pragmatic clinical trial. *Nutrients*, 11, 15. <https://doi.org/10.3390/nu11112778>
- SACN (Scientific Advisory Committee on Nutrition), 2016. Vitamin D and Health report. 304.
- Samaranayake D, Adikaram SGS, Atapattu N, Kendaragama K, Senevirathne JTN, Jayasekera HD and Wickramasinghe VP, 2020. Vitamin D supplementation in obese Sri Lankan children: a randomized controlled trial. *BMC Pediatrics*, 20, 426.
- Samelson EJ, Broe KE, Xu H, Yang L, Boyd S, Biver E, Szulc P, Adachi J, Amin S, Atkinson E, Berger C, Burt L, Chapurlat R, Chevalley T, Ferrari S, Goltzman D, Hanley DA, Hannan MT, Khosla S, Liu CT, Lorentzon M, Mellstrom D, Merle B, Nethander M, Rizzoli R, Sornay-Rendu E, Van Rietbergen B, Sundh D, Wong AKO, Ohlsson C, Demissie S, Kiel DP and Bouxsein ML, 2019. Cortical and trabecular bone microarchitecture as an independent predictor of incident fracture risk in older women and men in the Bone Microarchitecture International Consortium (BoMIC): a prospective study. *The Lancet Diabetes and Endocrinology*, 7, 34–43. [https://doi.org/10.1016/s2213-8587\(18\)30308-5](https://doi.org/10.1016/s2213-8587(18)30308-5)
- Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D and Nicholson GC, 2010. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA*, 303, 1815–1822. <https://doi.org/10.1001/jama.2010.594>
- Saneei P, Salehi-Abargouei A and Esmailzadeh A, 2013. Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. *Obesity Reviews*, 14, 393–404. <https://doi.org/10.1111/obr.12016>
- Sayers A, Fraser WD, Lawlor DA and Tobias JH, 2012. 25-Hydroxyvitamin-D3 levels are positively related to subsequent cortical bone development in childhood: findings from a large prospective cohort study. *Osteoporosis International*, 23, 2117–2128. <https://doi.org/10.1007/s00198-011-1813-9>
- SCF (Scientific Committee on Food), 2000. Guidelines of the Scientific Committee on Food for the development of tolerable upper intake levels for vitamins and minerals. Available online: [https://www.efsa.europa.eu/sites/default/files/efsa\\_rep/blobserver\\_assets/ndatolerableuil.pdf](https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf)
- SCF (Scientific Committee on Food), 2003. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of vitamin D. 35 pp. Available online: [https://www.efsa.europa.eu/sites/default/files/efsa\\_rep/blobserver\\_assets/ndatolerableuil.pdf](https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf)
- Schottker B, Jorde R, Peasey A, Thorand B, Jansen EH, Groot L, Streppel M, Gardiner J, Ordóñez-Mena JM, Perna L, Wilsgaard T, Rathmann W, Feskens E, Kampman E, Siganos G, Njolstad I, Mathiesen EB, Kubinova R, Pajak A, Topor-Madry R, Tamosiunas A, Hughes M, Kee F, Bobak M, Trichopoulou A, Boffetta P, Brenner H and Consortium on Health and Ageing: Network of Cohorts in Europe and the United States, 2014. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. *BMJ*, 348, g3656. <https://doi.org/10.1136/bmj.g3656>
- Schwartz JB, Kane L and Bikle D, 2016. Response of vitamin D concentration to vitamin D3 administration in older adults without sun exposure: a randomized double-blind trial. *Journal of the American Geriatrics Society*, 64, 65–72.
- Scragg R, 2020. The Vitamin D Assessment (ViDA) study - design and main findings. *The Journal of Steroid Biochemistry and Molecular Biology*, 198, 105562. <https://doi.org/10.1016/j.jsbmb.2019.105562>
- Scragg R, Khaw KT, Toop L, Sluyter J, Lawes CMM, Waayer D, Giovannucci E and Camargo CA Jr, 2018. Monthly high-dose vitamin D supplementation and cancer risk: a post hoc analysis of the vitamin D assessment randomized clinical trial. *JAMA Oncology*, 4, e182178. <https://doi.org/10.1001/jamaoncol.2018.2178>
- Seamans KM and Cashman KD, 2009. Existing and potentially novel functional markers of vitamin D status: a systematic review. *American Journal of Clinical Nutrition*, 89, 1997s–2008s. <https://doi.org/10.3945/ajcn.2009.27230D>

- Sempos CT, Durazo-Arvizu RA, Dawson-Hughes B, Yetley EA, Looker AC, Schleicher RL, Cao G, Burt V, Kramer H, Bailey RL, Dwyer JT, Zhang X, Gahche J, Coates PM and Picciano MF, 2013. Is there a reverse J-shaped association between 25-hydroxyvitamin D and all-cause mortality? Results from the U.S. nationally representative NHANES. *Journal of Clinical Endocrinology Metabolism*, 98, 3001–3009. <https://doi.org/10.1210/jc.2013-1333>
- SFA (Swedish Food Agency), 2015. Unpublished. Dietary Supplement Use Among the Swedish Adult Population. A study of the Determinants of Dietary Supplement Use and the Contribution of Vitamins and Minerals from Supplements to the total Micronutrient Intake. 99 pp.
- Shieh A, Ma C, Chun RF, Witzel S, Rafison B, Contreras HTM, Wittwer SJ, Swinkels L, Huijs T, Hewison M and Adams JS, 2017. Effects of cholecalciferol vs calcifediol on total and free 25-hydroxyvitamin D and parathyroid hormone. *Journal of Clinical Endocrinology and Metabolism*, 102, 1133–1140. <https://doi.org/10.1210/jc.2016-3919>
- Shirvani A, Kalajian TA, Song A, Allen R, Charoenngam N, Lewanczuk R and Holick MF, 2020. Variable genomic and metabolomic responses to varying doses of vitamin D supplementation. *Anticancer Research*, 40, 535–543.
- Siener R, 2021. Nutrition and kidney stone disease. *Nutrients*, 13, 1917.
- Smith LM, Gallagher JC and Suiter C, 2017. Medium doses of daily vitamin D decrease falls and higher doses of daily vitamin D3 increase falls: a randomized clinical trial. *Journal of Steroid Biochemistry and Molecular Biology*, 173, 317–322. <https://doi.org/10.1016/j.jsbmb.2017.03.015>
- Smith LM, Gallagher JC, Kaufmann M and Jones G, 2018. Effect of increasing doses of vitamin D on bone mineral density and serum N-terminal telopeptide in elderly women: a randomized controlled trial. *Journal of Internal Medicine*, 284, 685–693. <https://doi.org/10.1111/joim.12825>
- Sneve M, Figenschau Y and Jorde R, 2008. Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects. *European Journal of Endocrinology*, 159, 675–684.
- Snijder MB, van Schoor NM, Pluijm SM, van Dam RM, Visser M and Lips P, 2006. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. *The Journal of Clinical Endocrinology and Metabolism*, 91, 2980–2985. <https://doi.org/10.1210/jc.2006-0510>
- Steingrimsdottir L, Halldorsson TI, Siggeirsdottir K, Cotch MF, Einarsdottir BO, Eiriksdottir G, Sigurdsson S, Launer LJ, Harris TB, Gudnason V and Sigurdsson G, 2014. Hip fractures and bone mineral density in the elderly—importance of serum 25-hydroxyvitamin D. *PLoS ONE [Electronic Resource]*, 9, e91122. <https://doi.org/10.1371/journal.pone.0091122>
- Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, Carpenter J, Rücker G, Harbord RM, Schmid CH, Tetzlaff J, Deeks JJ, Peters J, Macaskill P, Schwarzer G, Duval S, Altman DG, Moher D and Higgins JP, 2011. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*, 343, d4002. <https://doi.org/10.1136/bmj.d4002>
- Stolzenberg-Solomon RZ, Jacobs EJ, Arslan AA, Qi D, Patel AV, Helzlsouer KJ, Weinstein SJ, McCullough ML, Purdue MP, Shu XO, Snyder K, Virtamo J, Wilkins LR, Yu K, Zeleniuch-Jacquotte A, Zheng W, Albanes D, Cai Q, Harvey C, Hayes R, Clipp S, Horst RL, Irish L, Koenig K, Le Marchand L and Kolonel LN, 2010. Circulating 25-hydroxyvitamin D and risk of pancreatic cancer: Cohort consortium vitamin D pooling project of rarer cancers. *American Journal of Epidemiology*, 172, 81–93. <https://doi.org/10.1093/aje/kwq120>
- Stos K, Wozniak A, Rychlik E, Ziolkowska I, Glowala A and Oltarzewski M, 2021. Assessment of food supplement consumption in Polish population of adults. *Frontiers of Nutrition*, 8. <https://doi.org/10.3389/fnut.2021.733951>
- Swanson CM, Srikanth P, Lee CG, Cummings SR, Jans I, Cauley JA, Bouillon R, Vanderschueren D, Orwoll ES and Nielson CM, 2015. Associations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D with bone mineral density, bone mineral density change, and incident nonvertebral fracture. *Journal of Bone and Mineral Research*, 30, 1403–1413. <https://doi.org/10.1002/jbmr.2487>
- Tangpricha V, 2012. Vitamin D in food and supplements. *American Journal of Clinical Nutrition*, 95, 1299–1300. <https://doi.org/10.3945/ajcn.112.039818>
- Tebben PJ, Singh RJ and Kumar R, 2016. Vitamin D-mediated hypercalcemia: mechanisms, diagnosis, and treatment. *Endocrine Reviews*, 37, 521–547. <https://doi.org/10.1210/er.2016-1070>
- Theodoratou E, Tzoulaki I, Zgaga L and Ioannidis JP, 2014. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ*, 348, g2035. <https://doi.org/10.1136/bmj.g2035>
- Thompson GR, Lewis B and Booth CC, 1966. Absorption of vitamin D<sub>3</sub>-<sup>3</sup>H in control subjects and patients with intestinal malabsorption. *Journal of Clinical Investigation*, 45, 94–102. <https://doi.org/10.1172/JCI105327>
- Thompson M, Aitken D, Balogun S, Cicuttini F and Jones G, 2021. Population vitamin D stores are increasing in tasmania, and this is associated with less bmd loss over 10 years. *Journal of Clinical Endocrinology and Metabolism*, 106, e2995–e3004. <https://doi.org/10.1210/clinem/dgab197>
- Totland TH, Melnæs BK, Lundberg-Hallén N, Helland-Kigen KM, Lund-Blix NA, Myhre JB, Wetting Johansen AM, Løken EB and Andersen LF (Universitetet i Oslo, Mattilsynet og Helsedirektoratet), 2012. Norkost 3 En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18–70 år, 2010–11. Oslo, Norway.
- Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hyppönen E, Berry J, Vieth R and Lanham-New S, 2012. Comparison of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *American Journal of Clinical Nutrition*, 95, 1357–1364. <https://doi.org/10.3945/ajcn.111.031070>



- Tripkovic L, Wilson LR, Hart K, Johnsen S, de Lusignan S, Smith CP, Bucca G, Penson S, Chope G, Elliott R, Hyppönen E, Berry JL and Lanham-New SA, 2017. Daily supplementation with 15 µg vitamin D(2) compared with vitamin D(3) to increase wintertime 25-hydroxyvitamin D status in healthy South Asian and white European women: a 12-wk randomized, placebo-controlled food-fortification trial. *American Journal of Clinical Nutrition*, 106, 481–490. <https://doi.org/10.3945/ajcn.116.138693>
- Uusi-Rasi K, Patil R, Karinkanta S, Kannus P, Tokola K, Lamberg A and H S, 2015. Exercise and vitamin D in fall prevention among older women: a randomized clinical trial. *JAMA Internal Medicine*, 175, 703–711. <https://doi.org/10.1001/jamainternmed.2015.0225>
- Vaes AMM, Tieland M, Toussaint N, Nilwik R, Verdijk LB, van Loon LJC and de Groot LCPGM, 2018a. Cholecalciferol or 25-hydroxycholecalciferol supplementation does not affect muscle strength and physical performance in prefrail and frail older adults. *Journal of Nutrition*, 148, 712–720. <https://doi.org/10.1093/jn/nxy024>
- Vaes AMM, Tieland M, de Regt MF, Wittwer J, van Loon LJC and de Groot L, 2018b. Dose-response effects of supplementation with calcifediol on serum 25-hydroxyvitamin D status and its metabolites: a randomized controlled trial in older adults. *Clinical Nutrition*, 37, 808–814. <https://doi.org/10.1016/j.clnu.2017.03.029>
- Valsta L, Kaartinen N, Tapanainen H, Männistö S and Sääksjärvi K (National Institute for Health and Welfare), 2018. Nutrition in Finland: The National FinDiet 2017 Survey.
- van Rossum CTM, Buurma-Rethans EJM, Dinnissen CS, Beukers MH, Brants HAM, Dekkers ALM and Ocké MC, 2020. The diet of the Dutch. In: National Institute for Public Health and the Environment (ed). Results of the Dutch National Food Consumption Survey 2012–2016. 79, Bilthoven, RIVM.
- Vanlint S, 2013. Vitamin D and obesity. *Nutrients*, 5, 949–956. <https://doi.org/10.3390/nu5030949>
- Vásquez-Caicedo AL, Bell S and Hartmann B (European Food Information Resource), 2008. Report on collection of rules on use of recipe calculation procedures including the use of yield and retention factors for imputing nutrient values for composite foods.
- Vieth R, 1999. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *American Journal of Clinical Nutrition*, 69, 842–856. <https://doi.org/10.1093/ajcn/69.5.842>
- Vieth R, Chan PCR and MacFarlane GD, 2001. Efficacy and safety of vitamin D-3 intake exceeding the lowest observed adverse effect level. *American Journal of Clinical Nutrition*, 73, 288–294.
- Viljakainen HT, Palssa A, Kärkkäinen M, Jakobsen J and Lamberg-Allardt C, 2006. How much vitamin D3 do the elderly need? *Journal of the American College of Nutrition*, 25, 429–435. <https://doi.org/10.1080/07315724.2006.10719556>
- Virtanen JK, Nurmi T, Aro A, Bertone-Johnson ER, Hyppönen E, Kroger H, Lamberg-Allardt C, Manson JE, Mursu J, Mantyselka P, Suominen S, Uusitupa M, Voutilainen A, Tuomainen TP and Hantunen S, 2022. Vitamin D supplementation and prevention of cardiovascular disease and cancer in the Finnish Vitamin D Trial: a randomized controlled trial. *American Journal of Clinical Nutrition*, 115, 1300–1310. <https://doi.org/10.1093/ajcn/nqab419>
- VKM (Norwegian Scientific Committee for Food and Environment (VKM)), 2022. Benefit and risk assessment of fish in the Norwegian diet 2022:17 Oslo, Norway. Available online: <https://vkm.no/english/riskassessments/allpublications/benefitandriskassessmentoffishinthenorwegiandiet.4.7b65040716afa427d7ec5d3a.html>
- Wacker M and Holick MF, 2013. Sunlight and Vitamin D: a global perspective for health. *Dermatoendocrinology*, 5, 51–108. <https://doi.org/10.4161/derm.24494>
- Wagner CL, Hulsey TC, Fanning D, Ebeling M and Hollis BW, 2006. High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. *Breastfeeding Medicine: The Official Journal of the Academy of Breastfeeding Medicine*, 1, 59–70.
- Wagner D, Hanwell HE, Schnabl K, Yazdanpanah M, Kimball S, Fu L, Sidhom G, Rousseau D, Cole DE and Vieth R, 2011. The ratio of serum 24,25-dihydroxyvitamin D(3) to 25-hydroxyvitamin D(3) is predictive of 25-hydroxyvitamin D(3) response to vitamin D(3) supplementation. *The Journal of Steroid Biochemistry and Molecular Biology*, 126, 72–77. <https://doi.org/10.1016/j.jsbmb.2011.05.003>
- Wagner H, Alvarsson M, Mannheimer B, Degerblad M and Ostenson CG, 2016. No effect of high-dose vitamin D treatment on beta-cell function, insulin sensitivity, or glucose homeostasis in subjects with abnormal glucose tolerance: a randomized clinical trial. *Diabetes Care*, 39, 345–352.
- Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C and Ashwell M, 2010. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. *Steroids*, 75, 477–488. <https://doi.org/10.1016/j.steroids.2010.02.012>
- Wallace RB, Wactawski-Wende J, O'Sullivan MJ, Larson JC, Cochrane B, Gass M and Masaki K, 2011. Urinary tract stone occurrence in the Women's Health Initiative (WHI) randomized clinical trial of calcium and vitamin D supplements. *American Journal of Clinical Nutrition*, 94, 270–277. <https://doi.org/10.3945/ajcn.110.003350>
- Wamberg L, Pedersen SB, Richelsen B and Rejnmark L, 2013. The effect of high-dose vitamin D supplementation on calciotropic hormones and bone mineral density in obese subjects with low levels of circulating 25-hydroxyvitamin d: results from a randomized controlled study. *Calcified Tissue International*, 93, 69–77.
- Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M and Vasan RS, 2008. Vitamin D deficiency and risk of cardiovascular disease. *Circulation*, 117, 503–511. <https://doi.org/10.1161/circulationaha.107.706127>

- Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anelli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidioglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasani RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Jarvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hypponen E and Spector TD, 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*, 376, 180–188. [https://doi.org/10.1016/S0140-6736\(10\)60588-0](https://doi.org/10.1016/S0140-6736(10)60588-0)
- Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J and Rhodes LE, 2018. Colour counts: sunlight and skin type as drivers of vitamin D Deficiency at UK Latitudes. *Nutrients*, 10. <https://doi.org/10.3390/nu10040457>
- Webb AR, Alghamdi R, Kift R and Rhodes LE, 2021. 100 years of vitamin D: dose-response for change in 25-hydroxyvitamin D after UV exposure: outcome of a systematic review. *Endocrine Connections*, 10, R248–r266. <https://doi.org/10.1530/ec-21-0308>
- WHO/IPCS (World Health Organization and International Programme on Chemical Safety), 2004. IPCS risk assessment terminology. World Health Organization. Available online: <https://apps.who.int/iris/handle/10665/42908>
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 1993. Biomarkers and Risk Assessment: Concepts and Principles. *Environmental Health Criteria*, 155, 86 pp. Available online. <https://apps.who.int/iris/handle/10665/39037>
- Wise SA, Camara JE, Sempos CT, Lukas P, Le Goff C, Peeters S, Burdette CQ, Nalin F, Hahm G, Durazo-Arvizu RA, Kuzsak AJ, Merkel J and Cavalier E, 2021. Vitamin D Standardization Program (VDSP) intralaboratory study for the assessment of 25-hydroxyvitamin D assay variability and bias. *The Journal of Steroid Biochemistry and Molecular Biology*, 212, 105917. <https://doi.org/10.1016/j.jsbmb.2021.105917>
- Wood AD, Secombes KR, Thies F, Aucott LS, Black AJ, Reid DM, Mavroei A, Simpson WG, Fraser WD and Macdonald HM, 2014. A parallel group double-blind RCT of vitamin D3 assessing physical function: is the biochemical response to treatment affected by overweight and obesity? *Osteoporosis International*, 25, 305–315. <https://doi.org/10.1007/s00198-013-2473-8>
- Yang Y, Wu F, Winzenberg T and Jones G, 2019. The association of vitamin D in youth and early adulthood with bone mineral density and microarchitecture in early adulthood. *Calcified Tissue International*, 104, 605–612. <https://doi.org/10.1007/s00223-019-00529-w>
- Yao P, Sun L, Lu L, Ding H, Chen X, Tang L, Xu X, Liu G, Hu Y, Ma Y, Wang F, Jin Q, Zheng H, Yin H, Zeng R, Chen Y, Hu FB, Li H and Lin X, 2017. Effects of genetic and nongenetic factors on total and bioavailable 25(OH)D responses to Vitamin D supplementation. *Journal of Clinical Endocrinology Metabolism*, 102, 100–110. <https://doi.org/10.1210/jc.2016-2930>
- Zhang M, Li P, Zhu Y, Chang H, Wang X, Liu W, Zhang Y and Huang G, 2015. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutrition & Metabolism (London)*, 12, 50. <https://doi.org/10.1186/s12986-015-0046-x>
- Zhang Y, Fang F, Tang J, Jia L, Feng Y, Xu P and Faramand A, 2019. Association between vitamin D supplementation and mortality: systematic review and meta-analysis. *BMJ*, 366, l4673. <https://doi.org/10.1136/bmj.l4673>
- Zhang R, Zhang Y, Liu Z, Pei Y, Xu P, Chong W, Hai Y, He L, He Y, Yu J, Wang J, Fang F and Peng X, 2022. Association between Vitamin D supplementation and cancer mortality: a systematic review and meta-analysis. *Cancers (Basel)*, 14. <https://doi.org/10.3390/cancers14153717>
- Zhu K, Bruce D, Austin N, Devine A, Ebeling PR and Prince RL, 2008a. Randomized controlled trial of the effects of calcium with or without vitamin D on bone structure and bone-related chemistry in elderly women with vitamin D insufficiency. *Journal of Bone and Mineral Research*, 23, 1343–1348. <https://doi.org/10.1359/jbmr.080327>
- Zhu K, Devine A, Dick IM, Wilson SG and Prince RL, 2008b. Effects of calcium and vitamin D supplementation on hip bone mineral density and calcium-related analytes in elderly ambulatory Australian women: a five-year randomized controlled trial. *Journal of Clinical Endocrinology and Metabolism*, 93, 743–749. <https://doi.org/10.1210/jc.2007-1466>
- Zhu K, Oddy WH, Holt P, Ping-Delfos WCS, Mountain J, Lye S, Pennell C, Hart PH and Walsh JP, 2017. Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass. *American Journal of Clinical Nutrition*, 106, 276–283. <https://doi.org/10.3945/ajcn.116.150524>
- Zittermann A and Prokop S, 2014. The role of vitamin D for cardiovascular disease and overall mortality. *Advances in Experimental Medicine and Biology*, 810, 106–119. [https://doi.org/10.1007/978-1-4939-0437-2\\_6](https://doi.org/10.1007/978-1-4939-0437-2_6)
- Zittermann A, Iodice S, Pilz S, Grant WB, Bagnardi V and Gandini S, 2012. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *American Journal of Clinical Nutrition*, 95, 91–100. <https://doi.org/10.3945/ajcn.111.014779>

## Abbreviations

|                            |   |
|----------------------------|---|
| 1,25(OH) <sub>2</sub> D    | 1,25-dihydroxyvitamin D, calcitriol                               |
| 1,24,25(OH) <sub>3</sub> D | 1,24,25-trihydroxyvitamin D                                       |
| 25(OH)D                    | 25-hydroxyvitamin D   |
| 25(OH)D <sub>3</sub>       | 25-hydroxyvitamin D <sub>3</sub> , 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                                       |

|       |   |
|-------|---|
| 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                 |

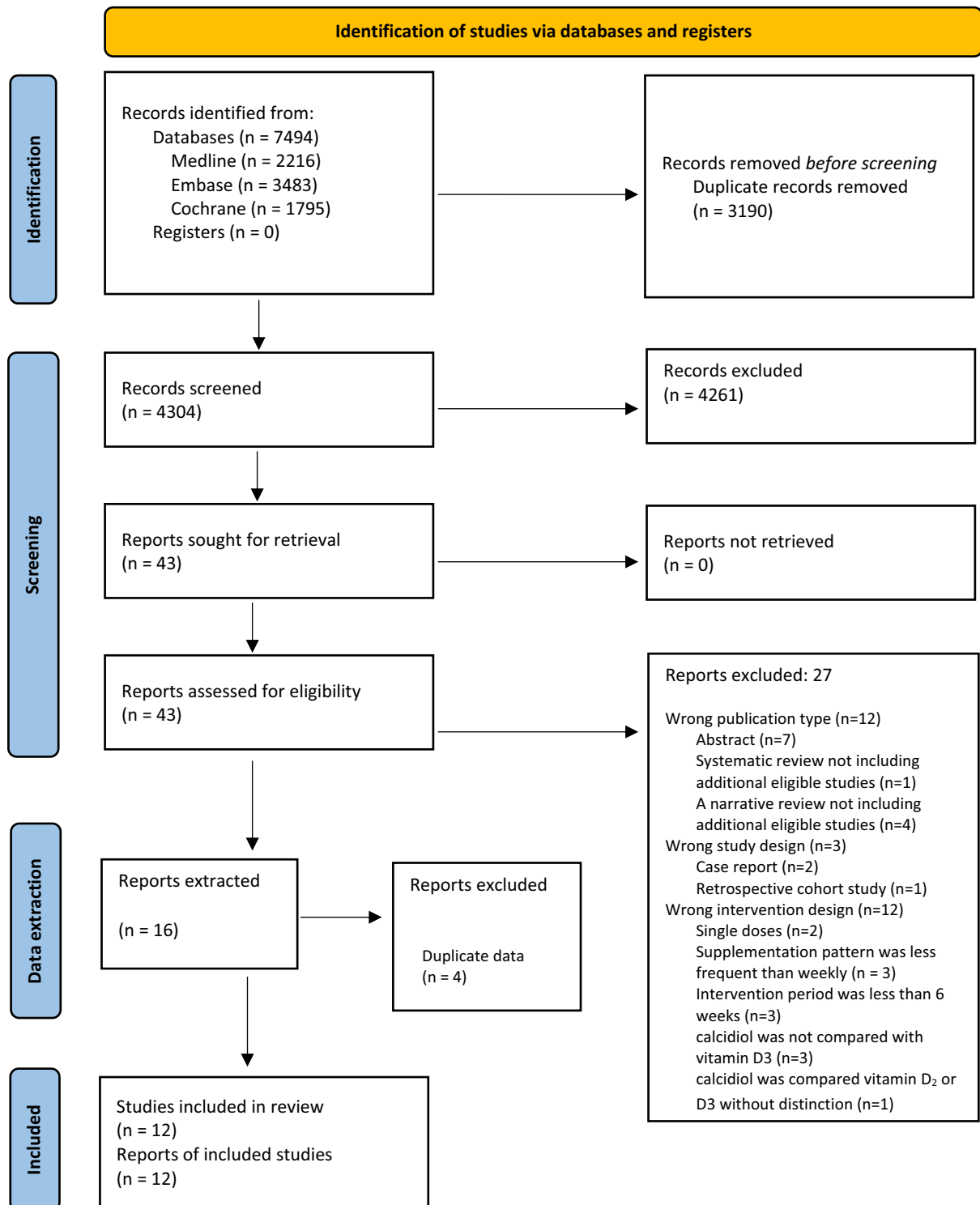
## Glossary

|                                 |  |
|---------------------------------|--|
| 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   |

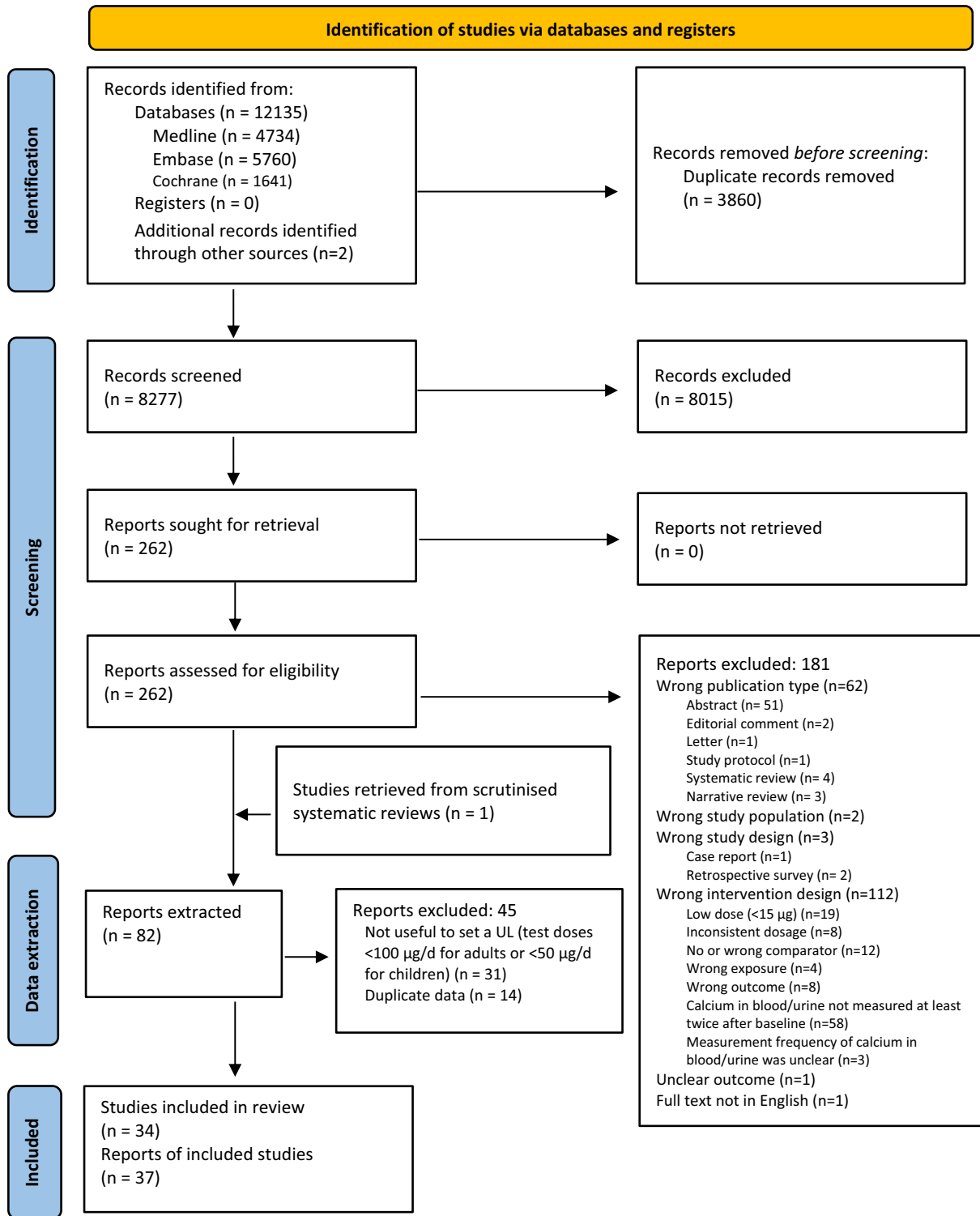


|  |   |
|--|---|
| Hazard                                       | Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population is exposed to that agent (WHO/IPCS, 2004; FAO/WHO, 2009)  |
| Lowest-observed-adverse-effect level (LOAEL) | 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.  |

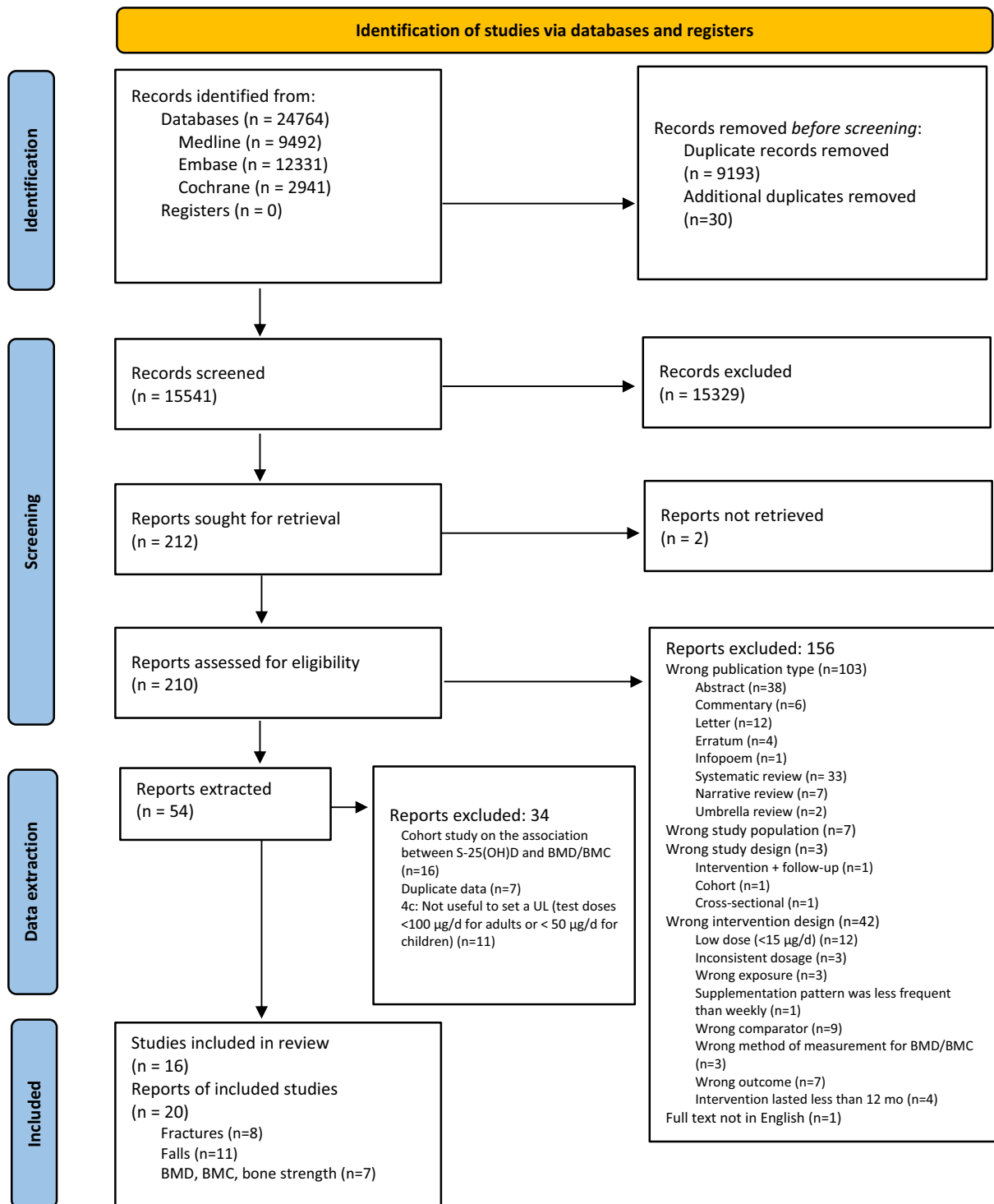
### Appendix A – Flow charts for the selection of studies



**Figure A.1:** Flow chart for the selection of studies on bioavailability of calcidiol and vitamin D<sub>3</sub>



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



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



## 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<sub>3</sub>

| References                   | Risk of bias domains <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|------------------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                              | 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; (--) : definitely 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 <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|-------------------------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                                     | Key Criteria                        |                           |                    | Other Criterion        |          |           |                     |                                    |                     |
|                                     | Randomization                       | Exposure characterization | Outcome assessment | Allocation concealment | Blinding | Attrition | Selective reporting | Other threats to internal validity |                     |
| <b>Children and adolescents</b>     |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| 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                   |
| <b>Pregnant and lactating women</b> |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| Enkmaa et al. 2019                  | ++                                  | +                         | -                  | ++                     | +        | ++        | ++                  | +                                  | 2                   |
| Hollis & Wagner 2004                | +                                   | +                         | NR                 | NR                     | NR       | --        | ++                  | -                                  | 2                   |

| References                           | Risk of bias domains <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|--------------------------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                                      | 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                   |
| <b>General adult population</b>      |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| 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 <sup>(c)</sup> | ++                                  | ++                        | +                  | ++                     | +        | +         | ++                  | ++                                 | 1                   |
| Gallagher et al. 2013 <sup>(c)</sup> | ++                                  | +                         | +                  | +                      | +        | +         | ++                  | +                                  | 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; ( - - ): definitely 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.3. Intervention studies on relationship between vitamin D intake and hypercalciuria according to population groups

| References                           | Risk of bias domains <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|--------------------------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                                      | Key Criteria                        |                           |                    | Other Criterion        |          |           |                     |                                    |                     |
|                                      | Randomization                       | Exposure characterization | Outcome assessment | Allocation concealment | Blinding | Attrition | Selective reporting | Other threats to internal validity |                     |
| <b>Children and adolescents</b>      |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| Lewis et al. 2013                    | ++                                  | ---                       | ++                 | ++                     | +        | +         | ++                  | +                                  | 2                   |
| <b>Lactating women</b>               |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| Hollis & Wagner 2004                 | +                                   | +                         | NR                 | NR                     | NR       | ---       | ++                  | -                                  | 2                   |
| Hollis et al. 2011                   | ++                                  | -                         | -                  | +                      | +        | -         | ++                  | +                                  | 2                   |
| Wagner et al. 2006                   | ++                                  | +                         | NR                 | ++                     | ++       | ---       | ++                  | +                                  | 2                   |
| <b>General adult population</b>      |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| 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 <sup>(c)</sup> | ++                                  | ++                        | ++                 | ++                     | +        | +         | ++                  | ++                                 | 1                   |
| Gallagher et al. 2013 <sup>(c)</sup> | ++                                  | +                         | +                  | +                      | +        | +         | ++                  | +                                  | 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; (---): definitely 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 <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|------------------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                              | 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                   |

| References            | Risk of bias domains <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|-----------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                       | 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; (---): definitely 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 <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|------------------------------|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|                              | 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; (---): definitely 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.6. Intervention studies on relationship between vitamin D intake and BMD, BMC, and Bone Strength

| References                                    | Risk of bias domains <sup>(a)</sup> |                           |                    |                        |          |           |                     |                                    | Tier <sup>(b)</sup> |
|---|-------------------------------------|---------------------------|--------------------|------------------------|----------|-----------|---------------------|------------------------------------|---------------------|
|   | Key Criteria                        |                           |                    | Other Criteria         |          |           |                     |                                    |                     |
|   | Randomization                       | Exposure characterization | Outcome assessment | Allocation concealment | Blinding | Attrition | Selective reporting | Other threats to internal validity |                     |
| <b>Children and adolescents<sup>(c)</sup></b> |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| 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                   |
| <b>General adult population</b>               |                                     |                           |                    |                        |          |           |                     |                                    |                     |
| 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; (--) : definitely 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.

## Appendix C – Evidence tables for the studies included

### C.1. Intervention studies on the relative bioavailability of calcidiol [25(OH)D<sub>3</sub>] compared to native vitamin D<sub>3</sub>

| Reference Study<br>Country Duration<br>Funding   | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results <sup>(a)</sup>  | RoB Tier |
|--|--|--|---|--|---|----------|
| Bischoff-Ferrari et al. (2012)<br>Switzerland<br>Latitude [assumed] 47.4° N<br>4 mo<br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>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 <sup>2</sup> , and ability of giving informed consent.<br><br>Exclusion criteria:<br>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<br><br>Age (y)<br>G1–G2: 63.45 ± 7.78<br>G3–G4: 59.48 ± 6.27<br><br>Serum 25(OH)D (ng/mL)<br>G1–G2:<br>14.18 ± 3.61 ng/mL<br>[35.45 ± 9.03 nmol/L]<br>G3–G4:<br>12.28 ± 4.08 ng/mL<br>[30.70 ± 10.2 nmol/L]<br><br>iPTH (pg/mL)<br>G1–G2: 54.87 ± 10.71<br>G3–G4: 63.22 ± 16.37<br><br>BMI (kg/m <sup>2</sup> )<br>G1–G2: 25.49 ± 3.38<br>G3–G4: 23.24 ± 3.22<br><br>Ethnicity: White<br><br>Smoking status:<br>Smokers were excluded<br><br>Alcohol use: NR<br><br>Health status:<br>Healthy population.<br><br>Season: NR | Doses<br>G1: vitamin D <sub>3</sub> 20 µg/d<br>G2: vitamin D <sub>3</sub> 140 µg/wk [=20 µg/d]<br>G3: calcidiol 20 µg/d<br>G4: calcidiol 140 µg/wk [=20 µg/d]<br><br>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.<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br><br>Pill count method:<br>Complete adherence stated. | Serum 25(OH)D: measured by HPLC–MS/MS<br><br>• time of blood collection after last dosage: NR<br><br>iPTH: measured by ECLIA | Data extracted: unclear<br><br>Serum 25(OH)D at 4 mo (ng/mL), mean ± SE<br>G1–G2: 30.99 ± 1.59 ng/mL [77.48 ± 3.98 nmol/L]<br>G3–G4: 69.47 ± 1.58 ng/mL [173.68 ± 3.95 nmol/L]<br><br>iPTH at 4 mo (pg/mL), mean ± SE<br>G1–G2: 51.68 ± 3.43<br>G3–G4: 43.00 ± 3.43 | <b>1</b> |

| Reference Study<br>Country Duration<br>Funding   | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results <sup>(a)</sup>   | RoB Tier |
|--|--|--|---|--|--|----------|
|  | study; use of high-dose vitamin D supplements >400 IU [ $>10 \mu\text{g}$ ]; use of high-dose calcium supplements >600 mg.<br><br>N participants randomised/completed/analysed<br>G1–G2: 10 October 2010<br>G3–G4: 10 October 2010   |  |   |  |  |          |
| Cashman et al. (2012)<br>Republic of Ireland<br>Latitude 51° N<br>10 wk<br>Mixed funding | RCT (parallel)<br><br>Inclusion criteria:<br>White men and women aged 50 years or more.<br><br>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)<br>G1: 63<br>G2: 62<br>G3: 50<br>G4: 42<br><br>Age (y)<br>All: $57.2 \pm 6.3$<br>NR across groups.<br><br>Serum 25(OH)D (nmol/L)<br>G1: $42.7 \pm 12.6$<br>G2: $49.7 \pm 16.2$<br>G3: $42.5 \pm 8.9$<br>G4: $38.2 \pm 9.9$<br><br>Serum iPTH (ng/mL)<br>G1: 65.6 (47.4–70.2)<br>G2: 47.3 (41.5–57.5)<br>G3: 58.6 (52.8–69.9)<br>G4: 57.9 (42.5–73.5)<br><br>BMI (kg/m <sup>2</sup> )<br>All: $28.3 \pm 4.8$<br>NR across groups.<br><br>Ethnicity: White | Doses<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 20 $\mu\text{g}/\text{d}$<br>G3: calcidiol 7 $\mu\text{g}/\text{d}$<br>G4: calcidiol 20 $\mu\text{g}/\text{d}$<br><br>Background vitamin D intake ( $\mu\text{g}/\text{d}$ )<br>G1: 6.5 (2.9–7.9)<br>G2: 7.6 (2.9–5.4)<br>G3: 5.1 (2.8–6.6)<br>G4: 4.4 (3.7–6.1)<br><br>Background calcium intake (mg/d)<br>G1: $970 \pm 503$<br>G2: $1114 \pm 494$<br>G3: $1008 \pm 415$<br>G4: $794 \pm 309$<br><br><u>Compliance</u><br><br>Pill count method (%)<br>All: 97.0 (92.0–100).<br>Stated to be similar across groups ( $P = 0.9$ ). | Serum 25(OH)D: measured by ELISA (OCTEIA)<br><br>Serum iPTH: measured by ELISA | <u>Data extracted: PP</u><br><br>Serum 25(OH)D at 5 wk/during intervention (nmol/L)<br>G1: $39.7 \pm 11.1$<br>G2: $64.1 \pm 9.5$<br>G3: $60.8 \pm 8.1$<br>G4: $98.1 \pm 20.5$<br><br>Serum 25(OH)D at 10 wk (nmol/L)<br>G1: $41.2 \pm 11.1$<br>G2: $69.0 \pm 8.7$<br>G3: $70.7 \pm 9.9$<br>G4: $134.6 \pm 26.0$<br><br>Serum iPTH at 10 wk (ng/mL)<br>G1: 65.8 (54.5–87.8)<br>G2: 44.2 (40.1–52.7)<br>G3: 52.7 (41.1–62.7)<br>G4: 40.5 (34.6–61.6) | <b>1</b> |

| Reference Study<br>Country Duration<br>Funding  | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results <sup>(a)</sup>   | 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.<br><br>N participants randomised/completed/analysed<br>G1: 16/16/16<br>G2: 14/13/13<br>G3: 14/14/14<br>G4: 14/13/12  | Smoking status: NR<br><br>Alcohol use: Excessive alcohol users were excluded.<br><br>Health status: Apparently healthy population.<br><br>Season: winter; 17–28th Jan to 21st Mar–8th Apr  |   |  |  |          |
| Catalano et al. (2015)<br>Italy<br>Latitude [assumed] 38.2° N<br>24 wk<br>Unclear funding | RCT (parallel)<br><br>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<br><br>Age (y)<br>G1: $59.46 \pm 6.12$<br>G2: $58.62 \pm 7.35$<br><br>Serum 25(OH)D (ng/mL)<br>G1: $20.35 \pm 5.46$ ng/mL [ $50.88 \pm 13.65$ nmol/L]<br>G2: $22.30 \pm 6.58$ ng/mL [ $55.75 \pm 16.45$ nmol/L]<br><br>Serum PTH: NR<br><br>BMI (kg/m <sup>2</sup> )<br>G1: $26.79 \pm 2.45$<br>G2: $27.48 \pm 2.81$<br><br>Ethnicity [assumed]: Majority Caucasian<br><br>Smoking status (%)<br>Current smoking: | Doses<br>G1: vitamin D <sub>3</sub> 140 µg/wk [=20 µg/d]<br>G2: calcidiol 140 µg/wk [=20 µg/d]<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br>NR | Serum 25(OH)D: method of measurement NR<br><br>• time of blood collection after last dosage: NR<br><br>Serum PTH: not measured | <u>Data extracted:</u> unclear<br><br>Serum 25(OH)D at 24 wk (ng/mL)<br>G1: $24.30 \pm 7.82$ ng/mL [ $60.75 \pm 19.55$ nmol/L]<br>G2: $50.33 \pm 15.45$ ng/mL [ $125.83 \pm 38.63$ nmol/L]<br><br>Serum PTH at 10 wk: NR | <b>3</b> |

| Reference Study<br>Country Duration<br>Funding  | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results <sup>(a)</sup>   | RoB Tier |
|---|--|---|---|---|--|----------|
|   | Exclusion criteria:<br>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.<br><br>N participants randomised/completed/analysed<br>G1: 28/NR/28<br>G2: 29/NR/29  | G1: 28<br>G2: 31<br><br>Alcohol use: NR<br><br>Health status (%)<br>Hypertension:<br>G1: 28<br>G2: 34<br>Diabetes:<br>G1: 36<br>G2: 44<br><br>Season:<br>Enrolment period Sept to Dec 2012.   |   |   |  |          |
| Corrado et al. (2021)<br><br>Italy<br><br>Latitude [assumed] 40.6° N<br><br>6 mo<br><br>Unclear funding [stated no external funding received] | RCT (parallel)<br><br>Inclusion criteria:<br>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 <sup>2</sup> and good health.<br><br>Exclusion criteria:<br>Taking drugs which could interfere with calcium and phosphate homeostasis; taking antihypertensive drugs, bisphosphonates, anticonvulsant, anticoagulant, | Sex: Females<br><br>Age (y)<br>G1: 63.4 ± 5.5<br>G2: 60.9 ± 8.1<br><br>Serum 25(OH)D (ng/mL)<br>G1: 12.5 ± 2.46<br>[31.25 ± 6.15 nmol/L]<br>G2: 13.3 ± 2.9<br>[33.25 ± 7.25 nmol/L]<br><br>Serum PTH (units unclear)<br>G1: 35.5 (4.1)<br>G2: 28.3 (8.08)<br>BMI (kg/m <sup>2</sup> )<br>G1: 23.8 ± 1.5<br>G2: 23.3 ± 1.2<br><br>Ethnicity: White | Doses<br>G1: vitamin D <sub>3</sub> 7000 IU/wk [175 µg/wk = 25 µg/d]<br>G2: calcidiol 7000 IU/wk [175 µg/wk = 25 µg/d]<br><br>The design also included two other groups treated with single dose of 300 000 IU [=7500 µg] of vitamin D <sub>3</sub> and monthly dose of 100 000 IU [=2500 µg] of vitamin D <sub>3</sub> . The groups were ignored here as supplementation pattern | Serum 25(OH)D: measured by CLIA (Diasorin LIASON kit [accredited VDSP])<br><br>• time of blood collection after last dosage: NR<br><br>Serum PTH: measured by EIA | <u>Data extracted:</u> unclear<br><br>Serum 25(OH)D <sub>3</sub> at 6 mo (nmol/L)<br>G1: 50.9 ± 4.52 [127.25 ± 11.3 nmol/L]<br>G2: 66.8 ± 3.98 [167.25 ± 9.95 nmol/L]<br><br>Serum PTH at 6 mo (units unclear)<br>G1: 34.2 (7.7)<br>G2: 31.2 (6.7) | <b>2</b> |



| Reference Study<br>Country Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed | Results <sup>(a)</sup> | 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.<br>Alcohol use: Abusers of alcohol were excluded.<br>Health status: Good health.<br>Season: Oct to Apr. | was less frequent than weekly.<br>Background vitamin D intake: NR<br>Background calcium intake: NR<br><u>Compliance</u><br>NR |                   |                        |          |

| Reference Study<br>Country Duration<br>Funding  | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results <sup>(a)</sup>  | RoB Tier |
|---|---|--|--|---|---|----------|
|   | N participants randomised/completed/analysed<br>G1: 31/27/27<br>G2: 30/26/26  |  |  |   |   |          |
| Graeff-Armas et al. (2020)<br>United Kingdom<br>Latitude 51° N<br>6 mo<br>Mixed funding | <p>RCT (parallel)</p> <p>Inclusion criteria:<br/>Healthy Caucasian men and postmenopausal women aged &gt;50 y, with a BMI of 20–32 kg/m<sup>2</sup> and willing to avoid direct sun exposure and restrict travel to sunny climates during the study period.</p> <p>Exclusion criteria:<br/>Taking vitamin D supplements (≤2 mo since last vitamin D supplement use), taking &gt;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</p> | <p>Sex (% females)<br/>G1: 59<br/>G2: 64<br/>G3: 61<br/>G4: 50</p> <p>Age (y)<br/>G1: 65.1 ± 9.3<br/>G2: 62.8 ± 8.4<br/>G3: 62.5 ± 6.8<br/>G4: 63.0 ± 7.3</p> <p>Serum 25(OH)D<sub>3</sub> (nmol/L)<br/>G1: 47.1 ± 15.0 (n = 21)<br/>G2: 48.0 ± 17.3<br/>G3: 49.5 ± 17.2<br/>G4: 48.5 ± 17.2</p> <p>Serum iPTH (pmol/L)<br/>G1: 3.91 ± 1.28<br/>G2: 4.12 ± 1.34<br/>G3: 3.81 ± 1.11<br/>G4: 3.99 ± 1.11</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 26.4 ± 3.1<br/>G2: 26.1 ± 2.3<br/>G3: 27.1 ± 3.1<br/>G4: 25.4 ± 2.5</p> <p>Ethnicity: Caucasian<br/>Smoking status: NR</p> | <p>Doses [labeled]<br/>G1: vitamin D<sub>3</sub> 20 µg/d<br/>G2: calcidiol 10 µg/d<br/>G3: calcidiol 15 µg/d<br/>G4: calcidiol 20 µg/d</p> <p>Analysed doses<br/>G1: vitamin D<sub>3</sub> 20.8 µg/d<br/>G2: calcidiol 10.8 µg/d<br/>G3: calcidiol 15.6 µg/d<br/>G4: calcidiol 21.8 µg/d</p> <p>Background vitamin D intake (µg/d): NR</p> <p>Background calcium intake (mg/d): NR</p> <p><u>Compliance</u><br/>Pill count method (%) &gt; 97 on average across groups and all visits.</p> | <p>Serum 25(OH)D<sub>3</sub>: measured by LC–MS/MS</p> <p>Serum iPTH: measured by CLIA (Roche Cobas; Roche)</p> | <p><u>Data extracted: PP</u></p> <p>Serum 25(OH)D<sub>3</sub> at 6 mo (nmol/L)<br/>G1: 82.8 ± 18.1 (n = 21)<br/>G2: 103 ± 22.9 (n = 19)<br/>G3: 130 ± 20.5<br/>G4: 153 ± 30.6</p> <p>Serum iPTH at 6 mo (pmol/L)<br/>G1: 4.77 ± 1.86<br/>G2: 4.31 ± 1.55<br/>G3: 4.00 ± 0.98<br/>G4: 4.00 ± 0.84 (n = 21)</p> | <b>1</b> |

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

| Reference Study<br>Country Duration<br>Funding  | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results <sup>(a)</sup>  | RoB Tier |
|---|--|--|--|---|---|----------|
|   | N participants randomised/completed/analysed<br>G1: 12 December 2012<br>G2: 12 December 2012<br>G3: 12 December 2012   | Alcohol use: Excessive alcohol users were excluded.<br><br>Health status: Healthy<br><br>Season: Mid-Oct 2006 to Mar 2007.   | Background calcium intake (mg/d):<br>806 ± 361<br>Measured 3 times during the intervention.<br><br><u>Compliance</u><br><br>Pill count method (%): 97  |   | G1: 43.5 (40.9, 46.4)<br>G2: 54.4 (51.1, 58.0)<br>G3: 63.8 (59.9, 67.9)<br><br>Serum iPTH after 6 wk (pmol/L)<br>G1: 2.6 (2.2, 3.0)<br>G2: 2.0 (1.7, 2.4)<br>G3: 2.2 (1.9, 2.6)<br><br>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)<br><br>Spain<br><br>Latitude 36.7° N<br><br>12 mo<br><br>Unclear funding | RCT (parallel)<br><br>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.<br><br>Exclusion criteria: NR<br><br>N participants randomised/completed/analysed<br>G1: 10/NR/NR<br>G2: 10/NR/NR<br>G3: 10/NR/NR | Sex: Females<br><br>Age (y)<br>All: 67 ± 6<br>NR across groups.<br><br>Serum 25(OH)D (nmol/L)<br>G1: 40.5 ± 4.7<br>G2: 37.2 ± 4.2<br>G3: 38.0 ± 3.7<br><br>Serum PTH (ng/L)<br>All: 57.2 ± 11.0<br>NR across groups.<br><br>BMI (kg/m <sup>2</sup> )<br>All: 26.4 ± 4<br>NR across groups.<br><br>Ethnicity [assumed]: Majority Caucasian<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: | Doses<br>G1: vitamin D <sub>3</sub> 20 µg/d<br>G2: calcidiol 20 µg/d<br>G3: calcidiol 266 µg/wk [=38 µg/d]<br><br>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.<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br>NR | Serum 25(OH)D: measured by HPLC and ultraviolet detection method<br><br>• time of blood collection after last dosage: NR<br><br>Serum PTH: measured by ECLIA (Roche Diagnostic) | <u>Data extracted</u> : unclear<br><br>Serum 25(OH)D at 6mo (nmol/L)<br>G1: 80.0 ± 2.0<br>G2: 161 ± 21.7<br>G3: 213.5 ± 80.0<br><br>Serum 25(OH)D at 12 mo (nmol/L)<br>G1: 86.2 ± 23.7<br>G2: 188.0 ± 24.0<br>G3: 233.0 ± 81.2<br><br>Serum 25(OH)D, change over 12 mo (nmol/L)<br>G1: 45.7 ± 19.5<br>G2: 150.8 ± 22.3<br>G3: 195 ± 79.3<br><br>Serum PTH at 6 mo (ng/L)<br>G1: 46.5 ± 11.2<br>G2: 38.7 ± 10.7<br>G3: 35.4 ± 8.4<br>Serum iPTH at 12 mo (ng/L)<br>G1: 41.6 ± 10.5<br>G2: 32.5 ± 8.8<br>G3: 29.0 ± 5.9 | <b>3</b> |

| Reference Study<br>Country Duration<br>Funding  | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results <sup>(a)</sup>  | RoB Tier |
|---|--|--|---|---|---|----------|
|   |  | Osteoporotic or osteopenic women?<br>Former stated in abstract, latter stated in the methods; method of diagnosis NR<br>Season: NR   |   |   |   |          |
| Okoye et al. (2022)<br>Italy<br>Latitude [assumed] 43.7° N<br>From hospital admission to 3 mo after discharge.<br>Unclear funding [stated no external funding received] | RCT (parallel)<br><br>Inclusion criteria:<br>Geriatric patients consecutively hospitalized in the Geriatric Unit of the University Hospital of Pisa for acute illness, with 25(OH)D <sub>3</sub> levels <30 ng/mL [ $<75$ nmol/L].<br><br>Exclusion criteria:<br>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)<br>G1: 56.5<br>G2: 59.1<br><br>Age (y):<br>G1: 84.9 ± 6.4<br>G2: 82.7 ± 6.7<br><br>Plasma 25(OH)D <sub>3</sub> (ng/mL)<br>G1: 16.8 ± 9.9 ng/mL [ $42.0 \pm 24.75$ nmol/L]<br>G2: 18.8 ± 13.3 ng/mL [ $47.0 \pm 33.25$ nmol/L]<br><br>PTH (ng/dL)<br>G1: 48.1 ± 39.6<br>G2: 60.7 ± 36.9<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 23.7 (7.2)<br>G2: 25.0 (5.6)<br><br>Ethnicity [assumed]:<br>Majority Caucasian<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status (%)<br>Arterial hypertension:<br>G1: 69.6 | Doses<br>G1: vitamin D <sub>3</sub> 437.5 µg/wk [=62.5 µg/d]<br>G2: calcidiol 140 µg/wk [=20 µg/d]<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br>Pill count method:<br>Complete adherence stated. | Plasma 25(OH)D <sub>3</sub> :<br>measured by HPLC–MS/MS<br><br>• time of blood collection after last dosage: NR<br><br>PTH:<br>method of measurement NR | <u>Data extracted:</u> unclear<br><br>Plasma 25(OH)D <sub>3</sub> at 3 mo (ng/mL)<br>G1: 30.7 ± 8.4 ng/mL [ $76.75 \pm 21.0$ nmol/L]<br>G2: 45.4 ± 9.8 ng/mL [ $113.5 \pm 24.5$ nmol/L]<br><br>PTH at 3 mo (ng/dL):<br>NR | <b>3</b> |



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

| Reference Study<br>Country Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed | Results <sup>(a)</sup> | RoB Tier |
|--|--|--|--|-------------------|------------------------|----------|
|  | <p>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.</p> <p>N participants randomised/completed/analysed<br/>G1: 33/NR/NR<br/>G2: 34/NR/NR</p> | <p>G1: 50 (38–85) or 64.1 ± 38.2<br/>G2: 104 (47–145) or 99.6 ± 51.2</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 27.5 (25.9–29.4)<br/>G2: 26.8 (24.4–28.6)</p> <p>Ethnicity[assumed]: Majority Caucasian</p> <p>Smoking status: NR</p> <p>Alcohol use: Excessive alcohol users were excluded.</p> <p>Health status:<br/>Number of comorbidities<br/>G1: 7 (5–10)<br/>G2: 7 (5–9)</p> <p>Season: NR</p> | <p>and, eventually, from supplements.</p> <p>Background vitamin D intake: NR</p> <p>Background calcium intake: NR</p> <p><u>Compliance</u></p> <p>Strategies to prove compliance were not implemented.</p> |                   |                        |          |

| Reference Study<br>Country Duration<br>Funding   | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results <sup>(a)</sup>  | RoB Tier |
|--|---|--|--|---|---|----------|
| Shieh et al. (2017)<br>USA<br>Latitude [assumed]<br>34.05° N<br>16 wk<br>Mixed funding | RCT (parallel)<br><br>Inclusion criteria:<br>Age ≥ 18 years and a baseline serum 25(OH)D level < 20 ng/mL [ $<50$ nmol/L]<br><br>Participants agreed to refrain from changing their dietary calcium intake and from taking self-prescribed calcium or vitamin D supplements for the study duration.<br><br>Exclusion criteria:<br>History of hypercalcaemia, hypercalciuria, nephrolithiasis, intestinal malabsorption, or dysregulated vitamin D metabolism (from underlying comorbidity or medication).<br><br>N participants randomised/completed/analysed<br>G1: 16/NR/NR<br>G2: 19/NR/NR | Sex: NR<br><br>Age (y)<br>G1: $36.9 \pm 12.7$<br>G2: $34.8 \pm 8.6$<br><br>Serum 25(OH)D (ng/mL)<br>G1: $16.2 \pm 3.7$ [ $40.5 \pm 9.25$ nmol/L]<br>G2: $17.0 \pm 2.5$ [ $42.5 \pm 6.25$ nmol/L]<br><br>Serum iPTH (pg/mL)<br>G1: $40.1 \pm 18.6$<br>G2: $34.6 \pm 13.9$<br><br>BMI (kg/m <sup>2</sup> )<br>G1: $25.7 \pm 6.1$<br>G2: $27.4 \pm 7.4$<br><br>Ethnicity (%)<br>White:<br>G1: 12.5<br>G2: 15.8<br>African American:<br>G1: 37.5<br>G2: 26.3<br>Asian American:<br>G1: 37.5<br>G2: 31.6<br>Hispanic/Latino:<br>G1: 12.5<br>G2: 26.3<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: Healthy<br><br>Season: NR | Doses<br>G1: vitamin D <sub>3</sub> 60 µg/d<br>G2: calcidiol 20 µg/d<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br>Pill count method (%)<br>G1: 90.1 on average<br>G2: 91.9 on average | Serum 25(OH)D: measured by CLIA (Diasorin LIAISON)<br><br>Serum iPTH: measured by CLIA (Cobas; Roche) | Data extracted: unclear<br><br>Serum 25(OH)D at 16 wk (ng/mL)<br>G1: $29.6 \pm 4.1$ [ $74.0 \pm 10.25$ nmol/L]<br>G2: $42.4 \pm 15.9$ [ $106.0 \pm 39.75$ nmol/L]<br><br>Serum iPTH (pg/mL) at 16 wk:<br>NR | <b>1</b> |

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

| Reference Study<br>Country Duration<br>Funding                                       | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results <sup>(a)</sup>  | 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.<br><br>N participants randomised/completed/analysed<br>G1: 26/25/25<br>G2: 26/24/24<br>G3: 26/26/26   | Prefrail:<br>G1: 96<br>G2: 96<br>G3: 81<br>Frail:<br>G1: 4<br>G2: 4<br>G3: 19<br><br>Season:<br>Dec 2014 to Dec 2015.<br><sup>2)</sup> model-adjusted means   |   |   |   |          |
| Vaes et al. (2018b)<br>the Netherlands<br>Latitude 51° N<br>24 wk<br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>65 years or older; a serum 25(OH)D <sub>3</sub> concentration between 25 and 50 nmol/L and a BMI between 20 and 35 kg/m <sup>2</sup> .<br><br>Exclusion criteria:<br>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)<br>G1: 64<br>G2: 43<br>G3: 40<br>G4: 44<br><br>Age (y)<br>G1: 78 ± 7.7<br>G2: 80 ± 7.3<br>G3: 79 ± 7.0<br>G4: 80 ± 7.0<br><br>Serum 25(OH)D <sub>3</sub> (nmol/L)<br>G1: 37.7 ± 7.0<br>G2: 43.4 ± 15.8<br>G3: 38.3 ± 10.5<br>G4: 38.6 ± 12.9<br><br>iPTH (pmol/L)<br>G1: 5.2 ± 1.9<br>G2: 5.7 ± 1.7 | Doses [labeled]<br>G1: vitamin D <sub>3</sub> 20 µg/d<br>G2: calcidiol 5 µg/d<br>G3: calcidiol 10 µg/d<br>G4: calcidiol 15 µg/d<br><br>Analysed doses<br>G1: vitamin D <sub>3</sub> 22.3 µg/d<br>G2: calcidiol 5.1 µg/d<br>G3: calcidiol 10.3 µg/d<br>G4: calcidiol 15.3 µg/d<br><br>Background vitamin D intake (µg/d)<br>G1: 3.7 ± 1.2<br>G2: 4.2 ± 1.6<br>G3: 3.3 ± 1.3<br>G4: 3.5 ± 1.5<br><br>Background calcium intake (mg/d) | Serum 25(OH)D <sub>3</sub> :<br>all samples were measured at the end of the study; measured by LC-MS/MS<br><br>iPTH:<br>measured by sandwich CLIA | <u>Data extracted: PP</u><br><br>Serum 25(OH)D <sub>3</sub> at 24 wk (nmol/L), mean (95% CI) <sup>4)</sup><br>G1: 71.6 (63.2, 80.0)<br>G2: 52.2 (44.4, 60.2)<br>G3: 88.7 (81.4, 96.1)<br>G4: 109.9 (102.5, 117.2)<br><br>iPTH at 24 wk (pmol/L), mean (95% CI) <sup>4)</sup><br>G1: 4.7 (4.1, 5.2)<br>G2: 5.1 (4.6, 5.6)<br>G3: 4.8 (4.3, 5.3)<br>G4: 3.9 (3.4, 4.4)<br><br><sup>4)</sup> model-predicted means; "The baseline level of the response variable and BMI were included as covariates in all models." | <b>1</b> |



| Reference Study<br>Country Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed | Results <sup>(a)</sup> | RoB Tier |
|--|--|--|---|-------------------|------------------------|----------|
|  | <p>interfere with vitamin D metabolism led to exclusion (e.g. thiazides, parathyroid hormone, bisphosphonates); consumed &gt;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.</p> <p>N participants randomised/completed/analysed<br/>                     G1: 14<sup>3)</sup>/12/11<br/>                     G2: 14<sup>3)</sup>/13/12<br/>                     G3: 15<sup>3)</sup>/15/14<br/>                     G4: 16<sup>3)</sup>/14/14</p> <p><sup>3)</sup> received intervention; one of the randomised participants did not receive treatment due to violation of eligibility criteria.</p> | <p>G3: 4.9 ± 1.3<br/>                     G4: 4.9 ± 1.8</p> <p>BMI (kg/m<sup>2</sup>)<br/>                     G1: 27.6 ± 3.5<br/>                     G2: 26.0 ± 4.4<br/>                     G3: 26.6 ± 3.7<br/>                     G4: 26.8 ± 3.9</p> <p>Ethnicity [assumed]:<br/>                     Majority Caucasian</p> <p>Smoking status (%)<br/>                     Non-smokers:<br/>                     G1: 43<br/>                     G2: 36<br/>                     G3: 40<br/>                     G4: 31<br/>                     Ex-smokers:<br/>                     G1: 50<br/>                     G2: 64<br/>                     G3: 60<br/>                     G4: 69<br/>                     Current smokers:<br/>                     G1: 7<br/>                     G2: 0<br/>                     G3: 0<br/>                     G4: 0</p> <p>Alcohol use (%)<br/>                     Light:<br/>                     G1: 71<br/>                     G2: 79<br/>                     G3: 93<br/>                     G4: 75<br/>                     Moderate:<br/>                     G1: 29<br/>                     G2: 21</p> | <p>G1: 985 ± 438<br/>                     G2: 1204 ± 487<br/>                     G3: 1041 ± 293<br/>                     G4: 1111 ± 386</p> <p><u>Compliance</u><br/>                     Pill count method (%)<br/>                     All: 97 on average.</p> |                   |                        |          |

| Reference Study<br>Country Duration<br>Funding | Design | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup> | Endpoint assessed | Results <sup>(a)</sup> | RoB Tier |
|--|--------|---|-----------------------------|-------------------|------------------------|----------|
|  |        | G3: 7<br>G4: 25<br>Excessive:<br>G1: 0<br>G2: 0<br>G3: 0<br>G4: 0<br><br>Health status: NR<br><br>Season:<br>26th Aug 2013 to 30th<br>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<sup>th</sup>-75<sup>th</sup> percentiles or the length between the percentiles.

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

| Reference<br>Study Country<br>Duration<br>Funding  | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results  | RoB<br>Tier |
|--|---|--|--|--|--|-------------|
| <b>Children and adolescents</b>  |   |  |  |  |  |             |
| Asghari et al. (2021)<br>Iran<br>Latitude 35.7° N<br>1 yr<br>Unclear [No external funding] | RCT (parallel)<br>Inclusion criteria:<br>Age 6–13 y; age- and sex-specific BMI Z-score ≥1.<br>Exclusion criteria:<br>Serious illness, vitamin D supplement use.<br>N participants, randomised/completed/analysed:<br>G1: 120/100 at 6mo, 92 at 12mo/120<br>G2: 127/106 at 6mo, 101 at 12mo/127<br>G3: 131/112 at 6mo, 108 at 12mo/131 | Sex (% girls)<br>G1: 50<br>G2: 48<br>G3: 45<br>Age (y)<br>G1: 9.2 ± 1.8<br>G2: 9.4 ± 1.6<br>G3: 9.4 ± 1.8<br>Serum 25(OH)D (ng/mL):<br>G1: 11.5 (8.9) ng/mL [28.8 (22.3) nmol/L]<br>G2: 11.7 (10.5) ng/mL [29.3 (26.3) nmol/L]<br>G3: 12.3 (10.2) ng/mL [30.8 (25.3)]<br>Assay: ECLIA (electrochemiluminescence immunoassay (Roche Diagnostics))<br>BMI (kg/m <sup>2</sup> )<br>G1: 23.2 ± 3.0<br>G2: 23.5 ± 3.5<br>G3: 23.3 ± 3.5<br>Ethnicity [assumed]:<br>Persian<br>Smoking status: NR<br>Alcohol use: NR | Vitamin D <sub>3</sub><br>Doses (labeled)<br>G1: vitamin D <sub>3</sub> 4 × 1000 IU/wk => 600 IU/d / 15 µg/d<br>G2: vitamin D <sub>3</sub> 1000 IU/d / 25 µg/d<br>G3: vitamin D <sub>3</sub> 2000 IU/d / 50 µg/d<br>Analysed vitamin D <sub>3</sub> doses<br>G2: 25.1 µg/d<br>G3: 51.1 µg/d<br>Background vitamin D intake (IU/d):<br>G1: 30.8 (71.6) IU/d [0.8 (1.8) µg/d]<br>G2: 43.2 (94.4) [1.1 (2.4) µg/d]<br>G3: 32.8 (86.0) [0.8 (2.2) µg/d]<br>Background calcium intake (mg/d):<br>G1: 574 (353)<br>G2: 605 (432)<br>G3: 610 (454)<br><u>Compliance</u> | Serum calcium:<br>- secondary outcome<br>- measured at baseline and at 6 and 12 mo<br>- 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.<br><br>Serum calcium at baseline (mg/dl), median (IQR)<br>G1: 9.9 (9.5–10.4)<br>G2: 10.1 (9.7–10.6)<br>G3: 9.9 (9.5–10.4)<br>Serum calcium at 6 mo (mg/dl), median (IQR)<br>G1: 10.4 (10.1–10.7)<br>G2: 10.3 (10–10.6)<br>G3: 10.5 (10–10.7)<br>Serum calcium at 12 mo (mg/dl), median (IQR)<br>G1: 10.4 (10.1–10.7)<br>G2: 10.3 (10.1–10.7)<br>G3: 10.3 (10.1–10.7) | <b>2</b>    |

| Reference<br>Study Country<br>Duration<br>Funding                                       | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results   | RoB<br>Tier |
|---|---|---|---|---|---|-------------|
|   |   | Health status: Overweight and obese<br>Season at recruitment (%):<br>Spring and summer:<br>G1: 50.8<br>G2: 50.4<br>G3: 49.6<br>Autumn and winter:<br>G1: 49.2<br>G2: 49.6<br>G3: 50.4   | Pill count method –<br>≥80% of pills consumed (%):<br>G1: 84.3 (76.9–91.8)<br>G2: 87.5 (78.5–96.0)<br>G3: 84.0 (76.3–92.6)<br><br>Serum 25(OH)D (ng/mL) at 12 mo:<br>G1: 23.11 (18.55–26.52) ng/mL [57.78 (46.38–66.3) nmol/L]<br>G2: 25.56 (21.27–29.58) ng/mL [63.9 (53.18–73.95) nmol/L]<br>G3: 28.63 (24.55–34.97) ng/mL [71.58 (61.38–87.43) nmol/L] |   |   |             |
| Belenchia et al. (2013)<br>USA<br>Latitude [assumed] 38.9° N<br>6 mo<br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>Aged 9 to 19 y; being at least at the 85th percentile for BMI.<br><br>Exclusion criteria:<br>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)<br>G1: 52<br>G2: 48<br><br>Age (y)<br>G1: 13.9 ± 2.4<br>G2: 14.6 ± 2.3<br><br>Serum 25(OH)D (ng/mL)<br>G1: 19.6 ± 7.9 [49 ± 19.75 nmol/L]<br>G2: 19.2 ± 6.3 [48 ± 15.75 nmol/L]<br>Assay: ELISA (Immunodiagnostik AG)<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 38.9 ± 6.7<br>G2: 39.5 ± 5.1 | Vitamin D <sub>3</sub><br><br>Doses<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d]<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br><br>Pill count method (%)<br>G1: 82<br>G2: 81<br><br>Serum 25(OH)D at 6 mo  | Serum calcium:<br>- secondary outcome<br>- measured at baseline and at 3 and 6 mo<br>- 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. | <b>2</b>    |

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



| Reference<br>Study Country<br>Duration<br>Funding | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed                   | Results   | RoB<br>Tier |
|---|---|---|---|-------------------------------------|---|-------------|
|   | N participants,<br>randomised/completed/<br>analysed:<br>G1: 66/63/63<br>G2: 64/59/59<br>G3: 65/63/63<br>G4: 64/61/61<br>G5: 64/58/58 | G4: 71.5 (30.4)<br>G5: 67.4 (30.1)<br><br>Ethnicity (%)<br>White: 48.9<br>Black: 51.1<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: Healthy<br>children<br><br>Season:<br>Enrolment in 2009 to 2010<br>and 2010 to 2011 during the<br>winter (Oct through Dec)<br>when serum 25(OH)D is at its<br>nadir. | Background vitamin D<br>intake (IU/d)<br>G1: 151 ± 96 IU/d<br>[3.8 ± 2.4 µg/d]<br>G2: 198 ± 140 IU/d<br>[5.0 ± 3.5 µg/d]<br>G3: 143 ± 111 IU/d<br>[3.6 ± 2.8 µg/d]<br>G4: 184 ± 160 IU/d<br>[4.6 ± 4.0 µg/d]<br>G5: 175 ± 101 IU/d<br>[4.4 ± 2.5 µg/d]<br><br>Background calcium<br>intake (mg/d)<br>G1: 837 ± 321<br>G2: 1000 ± 467<br>G3: 822 ± 375<br>G4: 914 ± 411<br>G5: 945 ± 378<br><br><u>Compliance</u><br><br>Pill count method –<br>vitamin D <sub>3</sub> /placebo (%)<br>All: 52.3<br><br>Serum 25(OH)D, change<br>over 12 wk (nmol/L),<br>mean ± SE<br>G1: -10.12 ± 2.86<br>G2: 5.54 ± 2.59<br>G3: 20.29 ± 2.61<br>G4: 37.57 ± 2.66<br>G5: 76.07 ± 2.95 |                                     |   |             |
| Maalouf et al.<br>(2008)                          | RCT (parallel)<br><br>Inclusion criteria:   | Sex (%)<br>Females: 49.4  | Vitamin D <sub>3</sub><br><br>Doses (labeled)   | Hypercalcaemia:<br>- safety measure | Elevated serum calcium,<br><i>n</i> of participants | <b>2</b>    |

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

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

| Reference<br>Study Country<br>Duration<br>Funding  | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results   | RoB<br>Tier |
|--|--|---|---|---|---|-------------|
|  | <p>who had fasting glucose concentrations of &lt;125 mg/dL and, in the case of postmenarchial girls, had a negative urine pregnancy test at the time of randomization.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 76/50/50<br/>G2: 74/48/47<br/>G3: 75/58/58</p>                      | <p>G1: 93.4<br/>G2: 94.6<br/>G3: 93.3<br/>Hispanic:<br/>G1: 2.6<br/>G2: 1.4<br/>G3: 9.3</p> <p>Smoking status: NR<br/>Alcohol use: NR<br/>Health status: Overweight or obese vitamin D-deficient children<br/>Season: NR</p>  | <p>Background calcium intake (mg/d)<br/>G1: 905 (701–1287)<br/>G2: 987 (647–1374)<br/>G3: 913 (599–1391)</p> <p><u>Compliance</u><br/>Pill count method and validated by an electronic medication event monitoring system – vitamin D<sub>3</sub> (%)<br/>All: 73 (at 3 and 6 mo)<br/>At 3 mo<br/>G1: 73<br/>G2: 68<br/>G3: 77<br/>At 6 mo<br/>G1: 73<br/>G2: 73<br/>G3: 73</p> |   |   |             |
| <p>Samaranayake et al. (2020)</p> <p>Sri Lanka</p> <p>Latitude [assumed] 6.9° N</p> <p>24 wk</p> <p>Public funding</p> | <p>(RCT parallel)</p> <p>Inclusion criteria:<br/>Obese children, who are identified to be having vitamin D deficiency (defined as 25(OH)D &lt; 20 ng/ml or &lt; 50 nmol/L) at a previously conducted cross-sectional study</p> <p>Exclusion criteria:<br/>Children who were suffering from any other</p> | <p>Sex (% Females)<br/>G1: 19.4<br/>G2: 33.3<br/>G3: 32.3</p> <p>Age (y)<br/>G1: 10.61 ± 1.83<br/>G2: 9.75 ± 2.26<br/>G3: 9.95 ± 2.02</p> <p>Serum 25(OH)D (ng/mL):<br/>G1: 15.47 ± 2.78 ng/mL [38.68 ± 6.95 nmol/L]<br/>G2: 14.92 ± 3.92 ng/mL [37.30 ± 9.80 nmol/L]</p> | <p>Vitamin D<sub>2</sub></p> <p>Doses<br/>G1: placebo<br/>G2: vitamin D<sub>2</sub> 2500 IU/wk [62.5 µg/wk = 8.9 µg/d]<br/>G3: vitamin D<sub>2</sub> 50 000 IU/wk [1250 µg/wk = 178.6 µg/d]</p> <p>Background vitamin D intake: NR<br/>Background calcium intake: NR</p>  | <p>Hypercalcaemia</p> <ul style="list-style-type: none"> <li>- safety measure</li> <li>- serum calcium measured at baseline, at 12 wk, and 6 mo</li> <li>- no cut-off provided</li> </ul> | <p>No adverse events were reported in the study population.<br/>Mean serum calcium decreased significantly in each group.</p> | <b>2</b>    |

| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed                   | Results  | RoB<br>Tier |
|---|--|---|--|-------------------------------------|--|-------------|
|   | <p>chronic condition or were on long-term medication were excluded.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 31/27/31<br/>G2: 33/25/33<br/>G3: 32/27/32</p> | <p>G3: 14.92 ± 3.04 ng/mL<br/>[37.30 ± 7.60 nmol/L]<br/>Assay: NR</p> <p>BMI Z-score<br/>G1: 2.66 ± 0.55<br/>G2: 2.83 ± 0.86<br/>G3: 2.72 ± 0.65</p> <p>Ethnicity (%)<br/>Sinhala:<br/>G1: 74.2<br/>G2: 69.7<br/>G3: 64.5<br/>Tamil:<br/>G1: 10.5<br/>G2: 9.1<br/>G3: 12.9<br/>Muslim<br/>G1: 16.1<br/>G2: 21.2<br/>G3: 19.4</p> <p>Smoking status: NR<br/>Alcohol use: NR<br/>Health status: Obese children<br/>Season: NR</p> | <p><u>Compliance</u></p> <p>Participants kept a written record of their compliance with the treatment.<br/>During the follow up visits the subjects were assessed for compliance to treatment<br/>Stated: Compliance was found to be satisfactory in all the subjects who completed the trial.</p> <p>Serum 25(OH)D at 24 wk (ng/mL):<br/>G1: 15.77 ± 3.43 ng/mL<br/>[39.43 ± 8.58 nmol/L]<br/>G2: 15.26 ± 3.675 ng/mL [38.15 ± 9.19 nmol/L]<br/>G3: 18.24 ± 5.77 ng/mL [45.60 ± 14.43 nmol/L]</p> |                                     |  |             |
| <b>Pregnant and lactating women</b>               |  |   |  |                                     |  |             |
| Enkhmaa et al. (2019)<br>Mongolia                 | RCT (parallel)<br>Inclusion criteria:<br>Pregnant women; ≥18 y;  | Sex: Females<br>Age (y)<br>G1: 28.3 ± 5.6   | Vitamin D <sub>3</sub><br>Doses  | Hypercalcaemia:<br>- safety measure | There were no instances of hypercalcaemia in any study arm at any timepoint. | <b>2</b>    |



| Reference<br>Study Country<br>Duration<br>Funding  | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results | RoB<br>Tier |
|--|---|--|---|--|---------|-------------|
| Latitude 50° N<br>From 12–<br>16 weeks’<br>gestation to 36–<br>40 weeks’<br>gestation<br>Mixed funding | 12–16 weeks pregnant;<br>planning to deliver at<br>Mandal Soum Hospital;<br>willing to forego vitamin D<br>supplements other than<br>the study capsules.<br><br>Exclusion criteria:<br>Current known seizure<br>disorder, renal failure,<br>parathyroid disease,<br>thyroid disease,<br>sarcoidosis, cancer, or<br>tuberculosis; history of<br>kidney stones; known<br>sensitivity to multivitamin<br>preparations;<br>already taking vitamin D<br>supplements containing<br>>600 IU/day [15 µg/d].<br><br>N participants,<br>randomised/completed/<br>analysed:<br>G1: 119/109/119<br>G2: 121/111/121<br>G3: 120/113/120 | G2: 28.5 ± 5.7<br>G3: 28.5 ± 5.4<br><br>Serum 25(OH)D (nmol/L)<br>G1: 18 ± 21<br>G2: 20 ± 24<br>G3: 20 ± 22<br>Assay: VIDAS <sup>®</sup> enzyme linked<br>fluorescent assay (ELFA,<br>Biomérieux)<br><br>Weight status (%)<br>Underweight (<18.5 kg/m <sup>2</sup> ):<br>G1: 0<br>G2: 1<br>G3: 0<br>Normal weight (18.5–24.9<br>kg/m <sup>2</sup> )<br>G1: 40<br>G2: 42<br>G3: 54<br>Overweight (25–29.9 kg/m <sup>2</sup> ):<br>G1: 40<br>G2: 42<br>G3: 29<br>Obese (≥30 kg/m <sup>2</sup> ):<br>G1: 20<br>G2: 15<br>G3: 17<br><br>Ethnicity [assumed]:<br>Mongol<br><br>Smoking status (%)<br>Never:<br>G1: 94<br>G2: 93 | G1: vitamin D <sub>3</sub> 600<br>IU/d [15 µg/d] + calcium<br>300 mg/d<br>G2: vitamin D <sub>3</sub> 2000<br>IU/d [50 µg/d] + calcium<br>300 mg/d<br>G3: vitamin D <sub>3</sub> 4000<br>IU/d [100 µg/d] +<br>calcium 300 mg/d<br><br>Vitamin D <sub>3</sub> content was<br>validated by Covance<br>Laboratories (Madison,<br>WI) using LC–MS to<br>ensure the capsules met<br>label claims at the outset<br>of the study.<br><br>Background vitamin D<br>intake: NR<br><br>Background calcium<br>intake: NR<br><br><u>Compliance</u><br><br>Pill count method –<br>taking at least 80% of<br>their assigned vitamin D<br>supplements (%)<br>G1: 88<br>G2: 89<br>G3: 87<br><br>Serum 25(OH)D at<br>36–40 weeks’ gestation<br>(nmol/L)<br>G1: 46 ± 21 | - serum calcium measured at<br>2 mo and at 36–40 weeks of<br>gestation<br>- defined as >2.6 mmol/L /<br>10.4 mg/dl<br><br>In any instances of<br>hypercalcaemia, the Data<br>Safety and Monitoring Board<br>was to be immediately<br>informed and the participant<br>was to discontinue the<br>supplement until the calcium<br>concentrations could be<br>retested. Any woman with<br>confirmed hypercalcaemia<br>was to be withdrawn from the<br>study. |         |             |

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

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

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

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



| Reference<br>Study Country<br>Duration<br>Funding | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed | Results   | RoB<br>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 ( $\geq 300$ mg/dl (3+ or 4+) based on urine dipstick; Hypertension ( $\geq 1$ systolic blood pressure reading $\geq 140$ mmHg and/or $\geq 1$ diastolic blood pressure reading $\geq 90$ 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<br>Ethnicity [assumed]: Bangladeshi<br>Smoking status: NR<br>Alcohol use: NR<br>Health status: Healthy pregnant women<br>Season: Between March 2014 and Sept 2015 | wk = 100 $\mu$ g/d] + calcium 500 mg/d<br>G5: prenatal vitamin D <sub>3</sub> 28 000 IU/wk [700 $\mu$ g/wk = 100 $\mu$ g/d] + postpartum vitamin D <sub>3</sub> 28 000 IU/wk [700 $\mu$ g/d] + calcium 500 mg/d<br>Background vitamin D intake: NR<br>Background calcium intake: NR<br><u>Compliance</u><br>Pill count method (%)<br>Prenatal; 100% of scheduled doses:<br>G1: 87.8<br>G2: 88.2<br>G3: 86.9<br>G4: 84.3<br>G5: 90.9<br>Postpartum; 100% of scheduled doses:<br>G1: 76.9<br>G2: 70.9<br>G3: 74.7<br>G4: 67.8<br>G5: 74.5<br>Maternal serum 25(OH)D at delivery (nmol/L)<br>G1: 24.30 $\pm$ 15.96 |                   | G3: 1<br>G4: 1<br>G5: 5<br>Possible hypercalcaemia, <i>n</i> of participants<br>G1: 7<br>G2: 10<br>G3: 13<br>G4: 17<br>G5: 16 |             |

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

| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed | Results | RoB<br>Tier |
|---|--|---|--|-------------------|---------|-------------|
| 6 mo<br>Public funding                            | Exclusion criteria:<br>Included pre-existing type I or II diabetes, hypertension, parathyroid disease, and uncontrolled thyroid disease.<br><br>N participants, randomised/completed/analysed:<br>All: 19/10/NR<br>G1: 10/NR/NR<br>G2: 9/NR/NR | G2: 85<br>Assay: HPLC and RIA techniques<br><br>BMI (kg/m <sup>2</sup> ): NR<br><br>Ethnicity (%)<br>African American:<br>G1: 11.1<br>G2: 11.1<br>White<br>G1: 66.7<br>G2: 88.9<br>Hispanic:<br>G1: 22.2<br>G2: 0<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: Healthy adults<br><br>Season: NR | Background vitamin D intake (IU/d)<br>G1: 273.6 ± 274.5 IU/d [6.8 ± 6.9 µg/d]<br>G2: 272.6 ± 114.5 [6.8 ± 2.9 µg/d]<br><br>Background calcium intake (mg/d):<br>G1: 1116.8 ± 587.3<br>G2: 1133.2 ± 286.7<br><br><u>Compliance</u><br><br>Pill count method – vitamin D <sub>3</sub> (%)<br>G1: ≥80<br>G2: ≥80<br><br>Serum 25(OH)D at 7 mo (nmol/L), mean<br>G1: 38.4 ng/mL [96.0 nmol/L]<br>G2: 58.8 ng/mL [147.0 nmol/L] |                   |         |             |

**General adult population**

|   |  |  |  |   |   |          |
|---|--|--|--|---|---|----------|
| Aloia et al. (2013)<br>USA<br>Latitude [assumed] 40.7° N<br>6 mo<br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>White, healthy, postmenopausal women.<br><br>Exclusion criteria:<br>Any chronic medical illness; osteoporosis; pregnancy; use of medications that influence bone metabolism or interfere with vitamin D metabolism; unexplained | Sex: Females<br><br>Age (y)<br>G1: 58.6 ± 6.7<br>G2: 60.0 ± 8.5<br>G3: 59.7 ± 7.1<br>G4: 57.6 ± 7.1<br><br>Serum 25(OH)D (nmol/L)<br>G1: 67 ± 17<br>G2: 66 ± 19<br>G3: 64 ± 16<br>G4: 69 ± 17<br>Assay: RIA (Diasorin) | Vitamin D <sub>3</sub><br><br>Doses<br>G1: double placebo<br>G2: placebo + calcium 1200 mg/d<br>G3: vitamin D <sub>3</sub> 100 µg/d + placebo<br>G4: vitamin D <sub>3</sub> 100 µg/d + calcium 1200 mg/d<br><br>Background vitamin D intake (IU/d) | Hypercalcaemia:<br>- safety measure<br>- serum calcium measured at baseline and at 15 and 28 weeks.<br>- no cut-off provided<br><br>Hypercalciuria:<br>- safety measure<br>- spot urine sample collected at baseline and at 15 and 28 weeks | <b>Hypercalcaemia</b><br>One incident of transient hypercalcaemia was noted in G4.<br><br><b>Hypercalciuria</b><br>Ca/Cr ratio >0.23:<br>The frequency of values exceeding this concentration was highest in G4: 10 instances vs 1–2 in the other groups. | <b>2</b> |
|---|--|--|--|---|---|----------|

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

| Reference Study Country Duration Funding | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results   | RoB Tier |
|--|---|---|--|--|---|----------|
|  | <p>ago; if present, hypertension and diabetes stable for the last three months.</p> <p>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.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 66/45/66<br/>G2: 66/47/66</p> | <p>BMI (kg/m<sup>2</sup>)<br/>G1: 27.0 (23.6–30.8)<br/>G2: 28.0 (24.9–31.2)</p> <p>Ethnicity: White</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> <p>Health status: Healthy population</p> <p>Season: NR</p> | <p>800 mg/d calcium in G1; assuming dietary intake of 800 mg/d calcium in G2.</p> <p>Analysed doses by corporation (Tischcon):<br/>G1: 710 IU [17.75 µg/d]<br/>G2: 12 069 IU [301.73 µg/d]</p> <p>Analysed doses by independent laboratory (Covance Laboratories)<br/>G1: 755 IU [18.88 µg/d]<br/>G2: 12 700 IU [317.5 µg/d]</p> <p>Background vitamin D intake: NR</p> <p>Background calcium intake (mg/d)<br/>G1: 900 (675–1214)<br/>G2: 878 (628–1114)</p> <p><u>Compliance</u></p> <p>Pill count method – vitamin D (%)<br/>G1: 87<br/>G2: 85</p> <p>Pill count method – calcium (%)<br/>G1: 84<br/>G2: 81</p> | <p>- 24-h urine calcium collected at baseline and at 3, 6, 9, and 12 mo.<br/>- defined as 24-h urine calcium excretion &gt;250 mg.</p> <p>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.</p> | <p>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.</p> <p>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.</p> <p><b>Hypercalcaemia over 1 yr, OR (95% CI)</b><br/>Serum calcium not corrected for albumin: G2 vs G1: 0.84 (0.25, 2.79)<br/>Serum calcium corrected for albumin: G2 vs G1: 0.74 (0.22, 2.4)</p> <p><b>Hypercalciuria over 1 yr, n of ≥1 event</b><br/>G1: 19<br/>G2: 34</p> |          |



| Reference<br>Study Country<br>Duration<br>Funding  | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results  | RoB<br>Tier |
|--|--|--|--|--|--|-------------|
|  |  |  | Serum 25(OH)D (ng/mL) at 1 year:<br>G1: 33.7 ± 6.1 ng/mL [84.3 ± 15.3 nmol/L]<br>G2: 86.6 ± 26.4 ng/mL [215.5 ± 66.0 nmol/L]<br><br>Calcium intake at 1 year (mg/d):<br>G1: 1843 (1728–1986)<br>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.<br>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.<br><br><b>Hypercalciuria over 1 yr, OR (95% CI)</b><br>G2 vs G1: 3.6 (1.39, 9.30)<br>[the OR does not refer to recurrent cases alone] |             |
| Billington et al. (2020)<br><br>Canada<br><br>Latitude [assumed] 51.0° N<br><br>3 y<br><br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>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.<br><br>Exclusion criteria: | Sex (% Females)<br>G1: 48.4<br>G2: 53.6<br>G3: 50.8<br><br>Age (y)<br>G1: 62.0 ± 4.2<br>G2: 62.7 ± 4.3<br>G3: 62.0 ± 4.1<br><br>Serum 25(OH)D (nmol/L)<br>G1: 76 ± 21<br>G2: 80 ± 20 | Vitamin D <sub>3</sub><br><br>Doses<br>G1: vitamin D <sub>3</sub> 400 IU/d [10 µg/d]<br>G2: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d]<br>G3: vitamin D <sub>3</sub> 10 000 IU/d [250 µg/d]<br><br>If dietary calcium intake was <1200 mg/day, a daily supplement | Hypercalcaemia:<br>- secondary outcome<br>- serum calcium measured at baseline and at 3, 6, 12, 18, 24, 30, and 36 mo<br>- defined as total serum calcium >2.55 mmol/L,<br>- participants were asked to discontinue the study intervention if repeat testing demonstrated persistent hypercalcaemia. | <b>Hypercalcaemia</b><br>Hypercalcaemia, <i>n</i> of events<br>G1: 0<br>G2: 4<br>G3: 12<br><i>P</i> = 0.02 for trend.<br><br>Hypercalcaemia, <i>n</i> of ≥1 event<br>G1: 0<br>G2: 4  | <b>2</b>    |

| Reference<br>Study Country<br>Duration<br>Funding | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results   | RoB<br>Tier |
|---|---|---|--|---|---|-------------|
|   | <p>Serum 25(OH)D &lt; 30 or &gt; 125 nmol/L; serum calcium &gt;2.5 or &lt;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.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 124/113/124<br/>G2: 125/115/125<br/>G3: 124/109/124</p> | <p>G3: 78 ± 18<br/>Assay: CLIA (Diasorin Liaison XL system)</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 27.7 ± 4.4<br/>G2: 27.8 ± 5.0<br/>G3: 27.2 ± 4.4</p> <p>Ethnicity [assumed]:<br/>Caucasian</p> <p>Current smokers (%)<br/>G1: 2.4<br/>G2: 1.6<br/>G3: 4.0</p> <p>Alcohol use: NR</p> <p>Health status:<br/>Healthy population</p> <p>Season: NR</p> | <p>containing either 300 mg or 600 mg elemental calcium was provided to approximate a total daily intake of 1200 mg.</p> <p>Participants starting calcium supplementation at baseline (%)<br/>G1: 75<br/>G2: 63.2<br/>G3: 73.4</p> <p>During the study, 32.7% of supplement takers discontinued taking the supplement and 18.2% of those not taking supplement initiated the supplementation.</p> <p>Background vitamin D intake<br/>G1: 166 ± 88 IU/d [4.2 ± 2.2 µg/d]<br/>G2: 178 ± 92 IU/d [4.5 ± 2.3 µg/d]<br/>G3: 188 ± 120 IU/d [4.7 ± 3.0 µg/d]</p> <p>Background calcium intake (mg/d)<br/>G1: 600 ± 303<br/>G2: 624 ± 279<br/>G3: 639 ± 344</p> | <p>Hypercalciuria:<br/>- secondary outcome<br/>- 24-h urine collected at baseline and at 12, 24, and 36 mo<br/>- 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<br/>- defined as 24-h urine calcium excretion &gt;7.5 mmol/day for participants of &lt;75 kg body weight; a weight-based cutoff &lt;0.1 mmol/kg/day was used for those &gt;75 kg<br/>- Ca/Cr ratio was served as a safety flag for the identification of significant hypercalciuria; a ratio of ≥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.</p> <p>Participants were asked to discontinue the study</p> | <p>G3: 11</p> <p>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.</p> <p><b>Hypercalciuria</b><br/>Hypercalciuria, <i>n</i> of events<br/>G1: 27</p> |             |

| Reference<br>Study Country<br>Duration<br>Funding   | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results   | RoB<br>Tier |
|---|--|---|---|--|---|-------------|
|   |  |   | <p><u>Compliance</u></p> <p>Adherence rate of vitamin D doses (%)<br/>G1: 99.6<br/>G2: 99.7<br/>G3: 99.1</p> <p>Serum 25(OH)D at 3 mo (nmol/L)<br/>G1: 76 ± 17<br/>G2: 114 ± 22<br/>G3: 187 ± 38</p>                      | intervention if repeat testing demonstrated persistent hypercalcaemia.   | <p>G2: 40<br/>G3: 56</p> <p>Hypercalciuria, <i>n</i> of ≥1 event<br/>G1: 21<br/>G2: 28<br/>G3: 38</p> <p>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.</p> |             |
| <p>Brohult et al. (1973)</p> <p>Sweden</p> <p>Latitude [assumed] 60.1° N</p> <p>1 yr</p> <p>Private funding</p> | <p>CT</p> <p>Inclusion criteria:<br/>Out-patients with definite rheumatoid arthritis.</p> <p>Exclusion criteria:<br/>Treatment with steroid, gold, or antimalaria therapy (the institution of such therapy was avoided as far as possible).</p> <p>N participants, randomised/completed/analysed:<br/>G1: 25/21/NR</p> | <p>Sex (% Females) 68</p> <p>Age (y); mean, range<br/>All: 52, 18–69<br/>G1: 53, NR<br/>G2: 51, NR</p> <p>Serum 25(OH)D: NR</p> <p>BMI: NR</p> <p>Ethnicity [assumed]:<br/>Caucasian</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> | <p>Vitamin D<sub>2</sub></p> <p>Doses<br/>G1: placebo<br/>G2: vitamin D<sub>2</sub> 100 000 IU/d [2500 µg/d]</p> <p>Background vitamin D intake: NR</p> <p>Background calcium intake: NR</p> <p><u>Compliance</u>: NR</p> | <p>Serum calcium</p> <ul style="list-style-type: none"> <li>- secondary outcome</li> <li>- serum calcium checked every other month</li> <li>- no cut-off for elevated levels provided</li> </ul> | <p>Serum calcium did not change significantly in either of the groups. One of the patients G2 had a serum calcium value of <b>7.0</b> mg/l [3.5 mmol/L] after 10 months' treatment; this was the only serum calcium value to exceed the normal upper limit.</p>   | <b>3</b>    |

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

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



| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results | RoB<br>Tier |
|---|--|--|--|---|---------|-------------|
|   | <p>a short physical performance battery score of <math>\leq 9</math> (out of a possible 12 points)</p> <p>Exclusion criteria:<br/>Active parathyroid disease; chronic kidney disease; nephrolithiasis; malignancy; liver disease; malabsorption; diabetes; unstable heart disease; severe osteoarthritis; neurodegenerative disease; vitamin D intake <math>&gt;400</math> IU/d or a 25OHD level <math>&lt; 22.5</math> or <math>&gt; 60</math> nmol/L; a calcium intake <math>&gt;1000</math> mg/d; abnormal serum calcium or 24-h urinary calcium <math>&gt;275</math> 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 <math>35^\circ</math> N; use of tanning beds, wheelchair, walker, and nasal oxygen</p> | <p>Caucasian<br/>G1: 92<br/>G2: 78</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> <p>Health status: Older mobility-limited women</p> <p>Season: NR</p> | <p><u>Compliance</u></p> <p>Pill count method:<br/>Was performed but results NR; stated that adherence to the intervention was high.</p> <p>Serum 25(OH)D at 4 mo (nmol/L)<br/>G1: <math>52.5 \pm 17.1</math><br/>G2: <math>80.0 \pm 11.5</math></p> | <p>normalised and serum calcium was normal, pills could be resumed, but repeat testing on pills was performed during the following 2 and 4 weeks.</p> |         |             |

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

| Reference<br>Study Country<br>Duration<br>Funding | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed     | Results   | RoB<br>Tier |
|---|---|---|---|-----------------------|---|-------------|
| Latitude 41.2° N<br>21 wk<br>Private funding      | <p>Exclusion criteria:<br/>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.</p> <p>N participants, randomised/completed/analysed:<br/>All: 67/58/62<br/>G1: NR/NR/22<br/>G2: NR/NR/20<br/>G3: NR/NR/20</p> | <p>Age (y)<br/>G1: 47.1 ± 12.5<br/>G2: 45.7 ± 12.6<br/>G3: 44.5 ± 12.9</p> <p>Serum 25(OH)D (ng/mL)<br/>G1: 20.3 ± 6.4<br/>[50.8 ± 16 nmol/L]<br/>G2: 26.5 ± 6.7<br/>[66.3 ± 16.8 nmol/L]<br/>G3: 23.2 ± 15.2<br/>[58 ± 38 nmol/L]</p> <p>Assay: RIA (DiaSorin)</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 36.7 ± 4.6<br/>G2: 36.1 ± 5.1<br/>G3: 37.9 ± 7.2</p> <p>Ethnicity: Caucasian</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> <p>Health status: Healthy women and men with BMI ≥30.0 kg/m<sup>2</sup></p> <p>Season: Winter</p> | <p>G2: vitamin D<sub>3</sub> 5000 IU/d [125 µg/d]<br/>G3: vitamin D<sub>3</sub> 10 000 IU/d [250 µg/d]</p> <p>Analysed vitamin D<sub>3</sub> doses<br/>G1: 911 IU [22.8 µg/d]<br/>G2: 5747 IU [143.7 µg/d]<br/>G3: 11 495 IU [287.4 µg/d]</p> <p>Background vitamin D intake (IU/d)<br/>G1: 207 ± 277 [5.2 ± 6.9 µg/d]<br/>G2: 203 ± 230 [5.1 ± 5.8 µg/d]<br/>G3: 279 ± 313 [7.0 ± 7.8 µg/d]</p> <p>Background calcium intake: NR</p> <p><u>Compliance</u><br/>Pill count method – vitamin D (%)<br/>All: 95 on average, range 94–97.<br/>Serum 25(OH)D change at 21 weeks (ng/mL)<br/>G1: 12.4 ± 9.7 [31 ± 24.3 nmol/L]<br/>G2: 27.8 ± 10.2 [69.5 ± 25.5 nmol/L]</p> | - no cut-off provided | were no hypercalcaemia events during the study. |             |

| Reference<br>Study Country<br>Duration<br>Funding  | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results   | RoB<br>Tier |
|--|--|---|---|--|---|-------------|
| Gallagher et al. (2012); Gallagher et al. (2014a)<br><b>VIDOS</b><br>USA<br>Latitude [assumed] 41.2° N<br>1 yr<br>Mixed funding<br>2012 for baseline info, 2014 for the outcomes | RCT (parallel)<br>Inclusion criteria:<br>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<br>Exclusion criteria:<br>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<br>Age (y)<br>G1: 66 ± 6.5<br>G2: 68 ± 8.6<br>G3: 68 ± 8.1<br>G4: 66 ± 7.4<br>G5: 66 ± 6.3<br>G6: 69 ± 7.7<br>G7: 66 ± 7.1<br>G8: 65 ± 6.1<br>Serum 25(OH)D (nmol/L)<br>G1: 37.7 ± 9.1<br>G2: 37.8 ± 10.8<br>G3: 39.0 ± 9.5<br>G4: 37.4 ± 10.2<br>G5: 38.2 ± 10.1<br>G6: 39.8 ± 8.2<br>G7: 37.2 ± 9.2<br>G8: 38.6 ± 9.1<br>Assay: RIA (Diasorin)<br>BMI (kg/m <sup>2</sup> )<br>G1: 31.1 ± 5.3<br>G2: 30.3 ± 5.4<br>G3: 28.2 ± 6.1<br>G4: 30.0 ± 5.4<br>G5: 30.4 ± 5.4<br>G6: 30.2 ± 5.7<br>G7: 29.7 ± 6.4<br>G8: 32.1 ± 6.2<br>Ethnicity: White<br>Smoking status (%) | G3: 50.7 ± 16.4<br>[126.8 ± 41 nmol/L]<br>Vitamin D <sub>3</sub><br>Doses (labeled)<br>G1: placebo + calcium <sup>2)</sup><br>G2: vitamin D <sub>3</sub> 400 IU/d [10 µg/d] + calcium <sup>2)</sup><br>G3: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + calcium <sup>2)</sup><br>G4: vitamin D <sub>3</sub> 1600 IU/d [40 µg/d] + calcium <sup>2)</sup><br>G5: vitamin D <sub>3</sub> 2400 IU/d [60 µg/d] + calcium <sup>2)</sup><br>G6: vitamin D <sub>3</sub> 3200 IU/d [80 µg/d] + calcium <sup>2)</sup><br>G7: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d] + calcium <sup>2)</sup><br>G8: vitamin D <sub>3</sub> 4800 IU/d [120 µg/d] + calcium <sup>2)</sup><br><sup>2)</sup> Calcium to maintain total intake between 1200 to 1400 mg/d (was based on a baseline 7-day food diary)<br>Analysed vitamin D <sub>3</sub> doses (mean of the capsules measured every 6 mo over 3 y)<br>G2: 503 IU [12.6 µg]<br>G3: 910 IU [22.8 µg]<br>G4: 1532 IU [38.3 µg]<br>G5: 2592 IU [64.8 µg]<br>G6: 2947 IU [73.8 µg]<br>G7: 4209 IU [105.2 µg] | Hypercalcaemia:<br>- safety measure<br>- serum calcium measured at baseline and at 3, 6, 9, and 12 mo<br>- 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]<br>- for poststudy analysis, defined as serum calcium level ≥ 10.3 mg/dL [2.75 mmol/L]; i.e. exceeding the upper reference limit<br>Hypercalciuria:<br>- safety measure<br>- 24-h urine calcium measured at baseline and at 3, 6, 9, and 12 months.<br>- during the study, defined as a 24-h urine calcium >400 mg [10 mmol/d] at any study visit<br>- for poststudy analysis, defined as a 24-h urine calcium >300 mg [7.5 mmol]<br>- for poststudy analysis, severe hypercalciuria defined as a 24-h urine calcium >400 mg [10 mmol] | <b>Hypercalcaemia</b><br>Serum calcium ≥2.75 mmol/L, <i>n</i> of events<br>G1: 3<br>G2: 2<br>G3: 1<br>G4: 5<br>G5: 6<br>G6: 1<br>G7: 1<br>G8: 1<br>Serum calcium ≥2.75 mmol/L, <i>n</i> of ≥1 event<br>G1: 1<br>G2: 1<br>G3: 1<br>G4: 4<br>G5: 4<br>G6: 1<br>G7: 1<br>G8: 1<br><b>Hypercalciuria</b><br>24-h urine calcium >7.5 mmol/d, <i>n</i> of events<br>G1: 8 | <b>1</b>    |

| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results  | RoB<br>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.<br><br>N participants, randomised/completed/analysed:<br>G1: 21/18/19<br>G2: 20/16/18<br>G3: 21/17/18<br>G4: 20/19/19<br>G5: 21/20/20<br>G6: 20/18/18<br>G7: 20/16/17<br>G7: 20/18/18 | Current:<br>G1: 19<br>G2: 10<br>G3: 5<br>G4: 20<br>G5: 5<br>G6: 15<br>G7: 0<br>G8: 10<br>Former:<br>G1: 33<br>G2: 35<br>G3: 33<br>G4: 40<br>G5: 38<br>G6: 20<br>G7: 50<br>G8: 45<br>Never:<br>G1: 48<br>G2: 55<br>G3: 62<br>G4: 40<br>G5: 57<br>G6: 65<br>G7: 50<br>G8: 45<br><br>Alcohol users (%)<br>G1: 33<br>G2: 35<br>G3: 57 | G8: 4937 IU [123.4 µg]<br><br>Background vitamin D intake (IU/d)<br>G1: 105 ± 61 IU/d [2.6 ± 1.5 µg/d]<br>G2: 98 ± 58 IU/d [2.5 ± 1.5 µg/d]<br>G3: 135 ± 70 IU/d [3.4 ± 1.8 µg/d]<br>G4: 125 ± 71 IU/d [3.1 ± 1.8 µg/d]<br>G5: 98 ± 55 IU/d [2.5 ± 1.4 µg/d]<br>G6: 109 ± 62 IU/d [2.7 ± 1.6 µg/d]<br>G7: 106 ± 83 IU/d [2.7 ± 2.1 µg/d]<br>G8: 137 ± 86 IU/d [3.4 ± 2.2 µg/d]<br><br>Background calcium intake (mg/d)<br>G1: 593 ± 182<br>G2: 606 ± 212<br>G3: 741 ± 247<br>G4: 754 ± 244<br>G5: 612 ± 190<br>G6: 725 ± 263<br>G7: 673 ± 324<br>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.<br><br>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<br>G3: 11<br>G4: 8<br>G5: 15<br>G6: 10<br>G7: 10<br>G8: 6<br><br>24-h urine calcium >7.5 mmol/d, n of ≥1 event<br>G1: 4<br>G2: 8<br>G3: 7<br>G4: 5<br>G5: 6<br>G6: 6<br>G7: 6<br>G8: 5<br><br>24-h urine calcium >10 mmol/d, n of ≥1 event<br>G1: 3<br>G2: 2<br>G3: 3<br>G4: 2<br>G5: 4<br>G6: 1<br>G7: 2<br>G8: 2<br><br>24-h urine calcium levels >10 mmol/d were |             |



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

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

| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup> | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results | RoB<br>Tier |
|---|--|---|--|---|---------|-------------|
|   | <p>calcium &lt;10.3 mg/dL (&gt;2.57 mmol/L) or 0.3 mg/dL (&gt;0.075 mmol/L) more than the upper limit of normal on 2 baseline tests; 24-h urine calcium &gt;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 &gt;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 (&gt;10 mg/d) for more than 6 months; were currently on anticonvulsants (phenytoin, phenobarbital), high-dose thiazide therapy (&gt;37.5 mg/d), and any drugs interfering with vitamin D metabolism; or the subjects were not able to give informed consent.</p> <p>N participants, randomised/completed/analysed:</p> | <p>Health status: Healthy<br/>Season: NR</p>          | <p>Background calcium intake (mg/d)<br/>G1: 540 ± 190<br/>G2: NR<br/>G3: 604 ± 282<br/>G4: 523 ± 197<br/>G5: 529 ± 231<br/>G6: NR<br/>G7: NR<br/>G8: 584 ± 210</p> <p><u>Compliance</u></p> <p>Pill count method – vitamin D (%)<br/>in Omaha: 91<br/>in Indiana: 81</p> <p>Pill count method – calcium (%)<br/>in Omaha: 79<br/>in Indiana: 70</p> <p>Serum 25(OH)D at 1 yr<br/>G1 vs other groups: significantly lower (visual presentation)</p> | <p>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.</p> |         |             |

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

| Reference<br>Study Country<br>Duration<br>Funding                            | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results   | RoB<br>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<br><br>N participants, randomised/completed/analysed:<br>G1: 149/135/135<br>G2: 148/140/140 | Health status (%)<br>Asthma/COPD:<br>G1: 10<br>G2: 6<br>Thyroid disease:<br>G1: 9<br>G2: 13<br>Arthrosis:<br>G1: 9<br>G2: 11<br>Other musculoskeletal disease:<br>G1: 11<br>G2: 15<br>Hypertension:<br>G1: 19<br>G2: 17<br>Hypercholesterolemia:<br>G1: 11<br>G2: 12<br>Previous osteoporotic fracture:<br>G1: 36<br>G2: 36<br><br>Season: NR | Serum 25(OH)D change at 1 year (nmol/L)<br>G1: 18.0 ± 18.9<br>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)<br>USA<br>Latitude 41.2° N<br>≈20 wk<br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>Men in good general health, who habitually consumed no more than one serving of milk/d and who did not take a vitamin supplement.<br><br>Exclusion criteria:  | Sex: Men<br><br>Age (y)<br>All: 38.7 ± 11.2<br><br>Serum 25(OH)D (nmol/L) based on analysed doses<br>G1: 70.2 ± 23.4<br>G2: 72.1 ± 16.0<br>G3: 69.3 ± 16.7<br>G4: 65.6 ± 24.2   | Vitamin D <sub>3</sub><br><br>Doses (labeled)<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 1000 IU/d /25 µg/d<br>G3: vitamin D <sub>3</sub> 5000 IU/d /125 µg/d<br>G4: vitamin D <sub>3</sub> 10 000 IU/d / 250 µg/d | Elevated serum calcium above the upper limits of normal:<br>- safety measure<br>- total serum calcium measured at each visit<br>- defined as >2.6 mmol/L | <b>Elevated serum calcium</b> , <i>n</i> of events<br>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. | <b>3</b>    |



| Reference<br>Study Country<br>Duration<br>Funding  | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed  | Results  | RoB<br>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).<br>N participants, randomised/completed/analysed:<br>All: 67/NR/NR | Assay: RIA<br>BMI (kg/m <sup>2</sup> )<br>All: 26.2 ± 2.4<br><br>Ethnicity [assumed]:<br>Caucasian<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: Healthy men<br><br>Season: Winter months of 2 successive years (late Oct to late Feb or early Mar of each year) | Analysed vitamin D <sub>3</sub> doses<br>G2: 836 IU / 20.9 µg/d<br>G3: 5500 IU / 137.5 µg/d<br>G4: 11 000 IU/d / 275 µg/d<br><br>Background vitamin D intake: NR<br>Background calcium intake: NR<br><br><u>Compliance</u><br><br>Pill count method – vitamin D <sub>3</sub> /placebo: NR<br><br>Serum 25(OH)D, change at equilibrium (nmol/L) based on analysed doses:<br>G1: -11.4 ± 17.7<br>G2: 12.0 ± 16.2<br>G3: 91.9 ± 37.6<br>G4: 159.4 ± 62.4 |  |  |             |
| Hin et al. (2016)<br><b>BEST-D</b><br>United Kingdom<br>Latitude [assumed] 51° N to 58° N<br>1 yr<br>Mixed funding | RCT (parallel)<br><br>Inclusion/exclusion criteria: Individuals who were ambulatory, living in the community and not currently taking more than 400 IU (10 µg) vitamin D <sub>3</sub> daily were eligible to participate.<br><br>N participants,  | Sex (% Females)<br>G1: 49.0<br>G2: 50.0<br>G3: 49.0<br><br>Age (y)<br>G1: 72 ± 6<br>G2: 72 ± 6<br>G3: 71 ± 6<br><br>Serum 25(OH)D (nmol/L)<br>G1: 47 ± 1.5<br>G2: 55 ± 2.2  | Vitamin D <sub>3</sub><br><br>Doses<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 2000 IU/d / 50 µg/d<br>G3: vitamin D <sub>3</sub> 4000 IU/d / 100 µg/d<br><br>Background vitamin D intake: NR<br><br>Background calcium intake (mg/d)  | Elevated plasma levels of albumin-corrected calcium:<br>- safety measure<br>- percentage of participants with calcium above the reference interval at 1, 6 and 12 months - defined as >2.55 mmol/L | <b>Elevated plasma levels of albumin-corrected calcium above the normal range, n of events</b><br><br>Stated: At randomization, albumin-corrected calcium was mildly elevated (>2.55 mmol/L) in 8 participants (6 who were subsequently allocated vitamin D <sub>3</sub> and | <b>2</b>    |

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

| Reference Study Country Duration Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results  | RoB Tier |
|--|--|--|---|---|--|----------|
| Mixed funding                            | <p>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<sup>2</sup> (22.5–42 kg/m<sup>2</sup> for Asian Americans)</p> <p>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] &lt;50 mL/min/1.73 m<sup>2</sup>), 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.</p> | <p>G2: 27.7 ± 10.2 ng/mL [69.3 ± 25.5 nmol/L]<br/>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)</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 32.1 ± 4.4<br/>G2: 32.0 ± 4.5</p> <p>Ethnicity (%)<br/>Asian:<br/>G1: 5.3<br/>G2: 5.5<br/>Black or African American<br/>G1: 26.0<br/>G2: 24.9<br/>White:<br/>G1: 66.5<br/>G2: 66.9<br/>Other<br/>G1: 2.3<br/>G2: 2.8<br/>Hispanic or Latino<br/>G1: 8.7<br/>G2: 9.9</p> <p>Smoking status: NR<br/>Alcohol use: NR</p> | <p>asked to limit calcium supplements to 600 mg/d</p> <p>Background vitamin D intake IU/d<br/>G1: 316 ± 397 IU/d [7.9 ± 9.9 µg/d]<br/>G2: 310 ± 401 IU/d [7.8 ± 10.0 µg/d]</p> <p>Background calcium intake (mg/d)<br/>G1: 107 ± 176<br/>G2: 100 ± 175</p> <p><u>Compliance</u><br/>Pill count method – vitamin D<sub>3</sub>/placebo (%)<br/>All: 84.1</p> | <p>and ≥ the upper level of normal plus 1 mg/dL</p> <p>If serum calcium (uncorrected for albumin concentration) was &gt; 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 &gt; 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.</p> <p>If the first measurement of serum calcium was &gt; 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.</p> <p>Hypercalciuria:<br/>- secondary outcomes<br/>- urine Ca/Cr ratio was measured at month 3, and annually thereafter<br/>- defined as &gt;0.375</p> | <p><b>Hypercalcaemia</b>, IRR (95% CI)<br/>G2 vs G1: 1.49 (0.42, 5.27)</p> <p><b>Hypercalciuria</b>, <i>n</i> of participants<br/>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</p> <p><b>Hypercalciuria</b>, IRR (95% CI)<br/>G2 vs G1: 0.99 (0.06, 15.86)</p> |          |

| Reference<br>Study Country<br>Duration<br>Funding   | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results   | RoB<br>Tier |
|---|---|---|---|---|---|-------------|
|   | N participants, randomised/completed/analysed:<br>G1: 1212/1119/1212<br>G2: 1211/1123/1211  | Health status: Overweight/obese persons with prediabetes<br><br>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)<br>Norway<br>Latitude [assumed] 69.6° N<br>1 yr<br>Mixed funding | (RCT parallel)<br><br>Inclusion criteria:<br>Males and females, 21–70 years old, with BMI between 28.0 and 47.0 kg/m <sup>2</sup> were included.<br>If the serum calcium was in the range 2.50–2.55 mmol/l, serum PTH had to be below 5.0 pmol/l.<br><br>Exclusion criteria:<br>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)<br>G1: 65.8<br>G2: 64.3<br>G3: 62.7<br><br>Age (y)<br>G1: 48.9 ± 11.0<br>G2: 47.6 ± 11.9<br>G3: 46.4 ± 11.3<br><br>Serum 25(OH)D (nmol/L)<br>G1: 53.2 ± 15.4<br>G2: 51.4 ± 18.4<br>G3: 54.5 ± 16.7<br><br>Assay: ECLIA, using an automated clinical chemistry analyser (Modular E170; Roche Diagnostics)<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 35.1 ± 3.8<br>G2: 34.4 ± 3.9<br>G3: 35.0 ± 4.1<br><br>Ethnicity [assumed]:<br>Caucasian | Vitamin D <sub>3</sub><br><br>Doses<br>G1: Double placebo + calcium 500 mg/d<br>G2: Vitamin D <sub>3</sub> 20 000 IU/wk [500 µg/wk = 71.43 µg/d] + placebo + calcium 500 mg/d<br>G3: Double vitamin D <sub>3</sub> 20 000 IU/wk [1000 µg/wk = 142.86 µg/d] + calcium 500 mg/d<br><br>Background vitamin D intake µg/d<br>G1: 8.9 ± 6.2<br>G2: 9.1 ± 7.0<br>G3: 9.0 ± 6.7<br><br>Background dietary calcium intake (mg/d)<br>G1: 955 ± 390<br>G2: 943 ± 438<br>G3: 922 ± 368 | Hypercalcaemia:<br>- safety measure<br>- serum calcium drawn after 3, 6, and 9 mo<br>- 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.<br><br>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:<br>"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 | <b>2</b>    |

| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results   | RoB<br>Tier     |
|---|--|---|--|---|---|-----------------|
|   | <p>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 &lt;1.0), subjects with serum calcium &gt;2.55 mmol/l, males with serum creatinine &gt;129 mmol/l, and females with serum creatinine &gt;104 mmol/l were not included.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 149/112/112<br/>G2: 143/106/106<br/>G3: 153/116/116</p> | <p>Smoking status (%)<br/>Current smokers:<br/>G1: 21.5<br/>G2: 21.7<br/>G3: 22.9</p> <p>Alcohol use: NR<br/>Health status: Healthy overweight and obese men and women</p> <p>Season: Summer and winter</p> | <p><u>Compliance</u></p> <p>Pill count method – vitamin D<sub>3</sub>/placebo (%)<br/>All: 95</p> <p>Pill count method - calcium (%)<br/>G1: 83<br/>G2: 85<br/>G3: 81</p> <p>Serum 25(OH)D at 12 mo (nmol/L)<br/>G1: 50.0 (20.3–99.8)<br/>G2: 87.8 (51.5–162.3)<br/>G3: 112.1 (46.7–193.4)</p> |   | <p>and, in retrospect, their baseline serum calcium and PTH levels, although not outside the present limits for inclusion, indicated a disturbed calcium metabolism.”).</p> <p>Serum calcium levels (mmol/L) in each hypercalcaemic cases by their respective groups<br/>G1: at screening 2.46; at 6 mo 2.60; retest 2.50<br/>G2: at screening 2.30; at 6 mo 2.62; retest 2.60<br/>G3: at screening 2.24; at 9 mo 2.61; retest 2.38<br/>G3: at screening 2.31; at 3 mo 2.61; retest 2.39<br/>G3: at screening 2.29; at 3 mo 2.61; retest 2.53</p> |                 |
| <p>Mastaglia et al. (2006)<br/>Argentina</p>      | <p>RCT (parallel)<br/>Inclusion criteria:</p>  | <p>Sex: Females<br/>Age (y)<br/>G1: 61.9 (54.5–67.0)</p>  | <p>Vitamin D<sub>2</sub><br/>Doses (labeled)<br/>G1: calcium 500 mg/d</p>  | <p>Hypercalcaemia:<br/>- safety measure<br/>- serum calcium measured at baseline and at 1, 2 and 3 mo</p> | <p><b>Hypercalcaemia</b><br/>No individual value in any of the three groups was above the upper limit of</p>  | <p><b>2</b></p> |



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

| Reference<br>Study Country<br>Duration<br>Funding                  | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results                            | RoB<br>Tier |
|--|---|--|--|---|------------------------------------|-------------|
| Latitude<br>[assumed]<br>40.7° N<br><br>8 wk<br><br>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 <sup>2</sup> , 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 m <sup>2</sup> , a history of coronary artery disease, diabetes mellitus, or a 10-year Framingham risk score > 10%.<br><br>Exclusion criteria: Taking >400 IU of vitamin D <sub>2</sub> or D <sub>3</sub> , 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 <sup>2</sup> .<br><br>N participants, randomised/completed/analysed:<br>All: NR/151/151 | Age (y)<br>G1: 47.4 ± 12.8<br>G2: 48.4 ± 11.3<br><br>Serum 25(OH)D (ng/ml):<br>G1: 14.1 ± 5.7<br>[35.25 ± 14.25 nmol/L]<br>G2: 13.4 ± 5.3<br>[33.5 ± 13.25 nmol/L]<br><br>Assay: CLIA (LIASON, Diasorin)<br><br>BMI (kg/m <sup>2</sup> ): NR<br><br>Ethnicity (%)<br>Blacks:<br>G1: 47<br>G2: 45<br>Other ethnicities: NR<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: Vitamin D-deficient; elevated risk for cardiovascular disease<br><br>Season: NR | G2: vitamin D <sub>3</sub> 5 x 10 000 IU/wk [5 x 250 µg/wk = 178.6 µg/d]<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br><br>Pill count method: NR<br>Serum 25(OH)D at 8 wk (ng/mL)<br>G1: 14.6 ± 6.2<br>[36.5 ± 15.5 nmol/L]<br>G2: 43.0 ± 12.3<br>[107.5 ± 30.75 nmol/L] | - measured at baseline and at 4 and 8 weeks<br>- cut-off for elevated levels defined as >10.5 mg/dl [ $>2.625$ mmol/L]. | all subjects throughout the study. |             |

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

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

| Reference<br>Study Country<br>Duration<br>Funding | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed   | Results  | RoB<br>Tier |
|---|--|---|---|---|--|-------------|
| Private funding                                   | <p>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.</p> <p>Exclusion criteria: Diagnosed with a pure physical urticaria, hereditary or acquired angioedema, hypercalcaemia (&gt;10.3 mg/dL [2.5757.49 mmol/L]), renal insufficiency (glomerular filtration rate &lt; 50 mL/min/1.73 m<sup>2</sup>), primary hyperparathyroidism, sarcoidosis, granulomatous disease, or malignancy or were pregnant or lactating.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 21/17/17<br/>G2: 21/21/21</p> | <p>Serum 25(OH)D (ng/mL), mean ± SE<br/>G1: 37.1 ± 3.4 ng/mL [92.8 ± 8.5 nmol/L]<br/>G2: 28.8 ± 2.2 ng/mL [72.0 ± 2.5 nmol/L]<br/>Assay: MS/MS</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 30.5 ± 6.32<br/>G2: 30.6 ± 9.42</p> <p>Ethnicity (%)<br/>White:<br/>G1: 90.4<br/>G2: 95.2<br/>African American:<br/>G1: 4.7<br/>G2: 4.7<br/>Asian:<br/>G1: 4.7<br/>G2: 0</p> <p>Smoking status (%)<br/>Current smokers:<br/>G1: 23.8<br/>G2: 28.5<br/>Former smokers:<br/>G1: 52.3<br/>G2: 47.6</p> <p>Alcohol users (%)<br/>G1: 42.8<br/>G2: 14.2</p> <p>Health status: Adult patients with physician-diagnosed</p> | <p>Background vitamin D intake: NR</p> <p>Background calcium intake: NR</p> <p><u>Compliance</u></p> <p>Pill count method – vitamin D<sub>3</sub> (%)<br/>All: &gt;80<br/>Stated: Excellent compliance, with only 1 subject in G1 showing &lt;80% compliance.</p> <p>Serum 25(OH)D at 12 wk (ng/mL), mean ± SE<br/>G1: 35.8 ± 2.3 ng/mL [89.5 ± 5.8 nmol/L]<br/>G2: 56.0 ± 3.9 ng/mL [140.0 ± 9.8 nmol/L]</p> | <p>- safety measure<br/>- spot urine for urine calcium was collected for safety end points<br/>- unclear at which time points.<br/>- defined as spot urine calcium &gt;30 mg/dL [7.49 mmol/L].</p> <p>Specific stopping rules and discontinuation of the study included a serum calcium level &gt; 10.3 mg/dL [2.575 mmol/L].<br/>Safety guidelines were implemented if the spot urine calcium level was &gt; 30 mg/dL.</p> | [7.49 mmol/L], which resolved on repeat measurement. |             |



| Reference<br>Study Country<br>Duration<br>Funding                                     | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results  | RoB<br>Tier |
|---|---|--|--|--|--|-------------|
|   |   | Chronic urticaria with or without angioedema<br><br>Season: NR   |  |  |  |             |
| Schwartz et al. (2016)<br>USA<br>Latitude [assumed] 37.7° N<br>16 wk<br>Mixed funding | (RCT parallel)<br><br>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 m <sup>2</sup> ), 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)<br>G1: 70.0<br>G2: 63.2<br>G3: 65.0<br>G4: 76.9<br><br>Age (y)<br>G1: 84.9 ± 8.7<br>G2: 85.9 ± 8.5<br>G3: 89.5 ± 6.6<br>G4: 90.1 ± 6.6<br><br>Serum 25(OH)D (ng/mL)<br>G1: 29.4 ± 10.4 ng/mL [73.5 ± 26.0 nmol/L]<br>G2: 28.9 ± 10.4 ng/mL [72.3 ± 26.0 nmol/L]<br>G3: 29.0 ± 9.9 ng/mL [72.5 ± 24.8 nmol/L]<br>G4: 32.3 ± 6.0 ng/mL [80.8 ± 15.0 nmol/L]<br><br>Assay:<br>LC-MS/MS (Mayo Clinical Laboratories, Rochester, MN).<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 27.5 ± 6.8<br>G2: 27.6 ± 5.3<br>G3: 27.0 ± 5.4<br>G4: 28.2 ± 5.7 | Vitamin D <sub>3</sub><br><br>Doses (labeled)<br>G1: vitamin D <sub>3</sub> 800 IU/d [20 µg/d]<br>G2: vitamin D <sub>3</sub> 2000 IU/d [50 µg/d]<br>G3: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d]<br>G4: vitamin D <sub>3</sub> 50 000 IU/wk [1250 µg/wk = 178.6 µg/d]<br><br>Analysed doses<br>G1: 858 ± 29 IU [21.5 ± 0.73 µg/d] and 861 ± 45 IU [21.5 ± 1.1 µg/d]<br>G2: 2467 ± 69 [61.7 ± 1.7 µg/d] and 2482 ± 73 IU [62.0 ± 1.8 µg/d]<br>G3: 4839 ± 202 [121.0 ± 5.1 µg/d] and 4807 ± 108 IU [120.2 ± 2.7 µg/d]<br>G4: 68 354 ± 2296 IU [1 708 ± 57.4 µg/d] and 57 542 ± 356 IU [1 439 ± 8.9 µg/d]; | Hypercalcaemia:<br>- safety measure<br>- unclear measurement frequency of albumin-adjusted serum calcium<br>- no cut-off provided.<br>However, chemistry panels were analysed at baseline, midstudy, and study end, probably including serum calcium | <b>Hypercalcaemia</b><br>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).<br><br>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. | <b>2</b>    |

| Reference<br>Study Country<br>Duration<br>Funding   | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed   | Results   | RoB<br>Tier |
|---|---|--|--|---|---|-------------|
|   | <p>than 2 months before entry.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 23/20/20<br/>G2: 20/19/19<br/>G3: 24/20/20<br/>G4: 14/13/13</p>  | <p>Ethnicity (%)</p> <p>White:<br/>G1: 95<br/>G2: 100<br/>G3: 100<br/>G4: 100</p> <p>Asian:<br/>G1: 5<br/>G2: 0<br/>G3: 0<br/>G4: 0</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> <p>Health status: Clinically stable long-term stay nursing home residents</p> <p>Season: NR</p> | <p>at study initiation and end, respectively.</p> <p>Background vitamin D intake: NR</p> <p>Background calcium intake: NR</p> <p><u>Compliance</u></p> <p>Pill count method – vitamin D<sub>3</sub> (%)<br/>All: 96 ± 7</p> <p>Serum 25(OH)D at 16 wk (ng/mL)</p> <p>G1: 33 ± 6 ng/mL [82.5 ± 15.0 nmol/L]<br/>G2: 34 ± 6 ng/mL [85.0 ± 15.0 nmol/L]<br/>G3: 43 ± 10 ng/mL [107.5 ± 25.0 nmol/L]<br/>G4: 61 ± 14 ng/mL [152.5 ± 35.0 nmol/L]</p> |   |   |             |
| <p>Shirvani et al. (2020)</p> <p>USA</p> <p>Latitude [assumed] 42.3° N</p> <p>6 mo</p> <p>Unclear funding</p> | <p>(RCT parallel)</p> <p>Inclusion criteria:<br/>Healthy young black and white adults with a BMI &lt; 30 kg/m<sup>2</sup> without disorders or medications affecting vitamin D metabolism.</p> <p>Exclusion criteria:</p> | <p>Sex (% Females)<br/>G1: 66.7<br/>G2: 61.5<br/>G3: 62.5</p> <p>Age (y)<br/>G1: 26.3 ± 2.0<br/>G2: 25.3 ± 2.1<br/>G3: 26.1 ± 2.0</p> <p>Serum 25(OH)D (ng/mL)</p>   | <p>Vitamin D<sub>3</sub></p> <p>Doses<br/>G1: vitamin D<sub>3</sub> 600 IU/d [15 µg/d]<br/>G2: vitamin D<sub>3</sub> 4000 IU/d [100 µg/d]<br/>G3: vitamin D<sub>3</sub> 10 000 IU/d [250 µg/d]</p> <p>Background vitamin D intake: NR</p>  | <p>Elevated serum calcium</p> <ul style="list-style-type: none"> <li>- safety measure</li> <li>- serum calcium measured at baseline and every 8 wk</li> <li>- defined as serum calcium &gt;10.5 mg/dL [2.625 mmol/L]</li> <li>- the participation of an individual who discovered to have elevated serum calcium</li> </ul> | <p>Serum calcium levels remained normal for all study participants and no untoward toxicity was observed.</p> | <b>2</b>    |

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

| Reference<br>Study Country<br>Duration<br>Funding                                     | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results  | RoB<br>Tier |
|---|--|--|--|--|--|-------------|
|   | N participants, randomised/completed/analysed:<br>All:73<br>G1: NR/33 ≥ 1 mo; 26 at ≥3 mo; 15 at 5 mo/NR<br>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)<br>BMI: NR<br>Ethnicity (%)<br>White:<br>G1: 66.6<br>G2: 71.4<br>Black:<br>G1: 6.1<br>G2:10.7<br>Asian:<br>G1: 27.3<br>G2: 17.9<br>Smoking status: NR<br>Alcohol use: NR<br>Health status: Healthy men and women.<br>Season: Winter and summer; the study began between Jan and Feb. | Background calcium intake: NR<br><u>Compliance</u><br>Vials that contained the vitamin D <sub>3</sub> solutions were collected and subjects were given fresh vials: NR<br>Peaked serum 25(OH)D at 3 mo (nmol/L)<br>G1: 68.7 ± 16.9<br>G2: 96.4 ± 14.6<br>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<br>- 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)<br>Sweden<br>Latitude [assumed] 59.3° N<br>8 wk<br>Mixed funding | RCT (parallel)<br>Inclusion criteria:<br>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)<br>G1: 50.0<br>G2: 42.9<br>Age (y)<br>G1: 67.0 (64.7–68.5)<br>G2: 67.6 (63.4–68.8)<br>Serum 25(OH)D (nmol/L)<br>G1: 47 (42–53)<br>G2: 42 (35–55)<br>Serum 25(OH)D (nmol/L), season-adjusted   | Vitamin D <sub>3</sub><br>Doses<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 30 000 IU µg/wk [750 µg/wk = 107.1 µg/d]<br>Background vitamin D intake: NR<br>Background calcium intake: NR  | Hypercalcaemia:<br>- secondary outcome<br>- serum calcium measured at baseline and at 4 and 8 wk<br>- defined as ionized serum calcium >1.35 mmol/L.               | No events of hypercalcaemia occurred in the study.   | 2           |

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



| Reference<br>Study Country<br>Duration<br>Funding | Design  | Subject characteristics at<br>baseline <sup>(a)</sup> | Intervention <sup>(a)</sup> | Endpoint assessed | Results | RoB<br>Tier |
|---|---|---|-----------------------------|-------------------|---------|-------------|
|   | <p>&gt;1.35 mmol/L;<br/>6) hyperphosphatemia at screening, defined as s-phosphate &gt;1.5 mmol/L;<br/>7) sarcoidosis or other granulomatous disease;<br/>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 &gt;133 mmol/L for males and &gt; 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 <math>\geq</math> 180 mmHg and/or diastolic</p> |   |                             |                   |         |             |

| Reference<br>Study Country<br>Duration<br>Funding  | Design   | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed  | Results   | RoB<br>Tier |
|--|--|---|--|--|---|-------------|
|  | <p>blood pressure <math>\geq</math> 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.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 22/21/21<br/>G2: 22/22/22</p> |   |  |  |   |             |
| <p>Wamberg et al. (2013)</p> <p>Denmark<br/>Latitude [assumed] 56.1° N</p> <p>26 wk</p> <p>Unclear funding</p> | <p>(RCT parallel)</p> <p>Inclusion criteria:<br/>Women not planning pregnancy and reporting the use of safe contraception.</p> <p>Exclusion criteria:<br/>A history of diabetes, a fasting plasma glucose [7.0 mmol/L,</p>   | <p>Sex: % (Females)<br/>G1: 73<br/>G2: 69</p> <p>Age (y)<br/>G1: 41.2 <math>\pm</math> 6.8<br/>G2: 39.5 <math>\pm</math> 8.0</p> <p>Serum 25(OH)D (nmol/L)<br/>G1: 34.6 <math>\pm</math> 10.3<br/>G2: 34.5 <math>\pm</math> 10.8</p> <p>Assay: LC-MS/MS</p> | <p>Vitamin D<sub>3</sub></p> <p>Doses<br/>G1: placebo<br/>G2: vitamin D<sub>3</sub> 7000 IU (175 <math>\mu</math>g/d)</p> <p>Background vitamin D intake (<math>\mu</math>g/d)<br/>G1: 1.7 (1.4–3.1)<br/>G2: 2.1 (1.5–4.0)</p> | <p>Hypercalcaemia:<br/>- safety measure<br/>- 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<br/>- reference range for serum ionized calcium defined as 1.18–1.32 mmol/L</p> | <p>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.</p> | <b>1</b>    |

| Reference<br>Study Country<br>Duration<br>Funding | Design  | Subject characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed | Results | RoB<br>Tier |
|---|---|---|--|-------------------|---------|-------------|
|   | <p>hypercalcaemia, or impaired renal plasma creatinine [130 μmol/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.</p> <p>N participants, randomised/completed/analysed:<br/>G1: 26/21/NR<br/>G2: 26/22/NR</p> | <p>BMI (kg/m<sup>2</sup>)<br/>G1: 35.0 ± 3.2<br/>G2: 36.1 ± 3.4</p> <p>Ethnicity [assumed]:<br/>Caucasian</p> <p>Smoking status (%)<br/>Current smokers:<br/>G1: 19<br/>G2: 27<br/>Ex-smokers:<br/>G1: 27<br/>G2: 31<br/>Non-smokers<br/>G1: 54<br/>G2: 42</p> <p>Alcohol use: Alcohol abusers were excluded.</p> <p>Health status: Healthy obese men and women</p> <p>Season: NR<br/>(Recruited from February 2010 until May 2011)</p> | <p>Background dietary calcium intake (mg/d)<br/>G1: 936 ± 389<br/>G2: 992 ± 400</p> <p><u>Compliance</u><br/>Pill count method – vitamin D<sub>3</sub>/placebo (%)<br/>G1: 94 ± 8<br/>G2: 95 ± 6</p> <p>Serum 25(OH)D at 26 wk (nmol/L)<br/>G1: 46.8 ± 21.2<br/>G2: 110 ± 17.3</p> |                   |         |             |

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

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

| Reference Study<br>Country<br>Duration<br>Funding  | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|--|---|---|--|---|---|----------|
| Appel et al. (2021)<br><b>STURDY</b><br>USA<br>Latitude 39° N<br>2 y<br>(median 22.0 mo, range 0.4–31.3 mo)<br>Mixed funding | RCT (Bayesian response-adaptive in dose-finding stage; parallel in best-dose stage)<br><br>Inclusion criteria:<br>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).<br><br>Exclusion criteria:<br>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.<br><br>Persons using 1000 IU/d [25 µg/d] or less of supplemental vitamin D had | Sex (% females)<br>G1: 43.0<br>G2: 47.1<br>G3: 42.6<br>G4: 40.6<br><br>Age (y)<br>G1: 77.5 ± 5.6<br>G2: 76.4 ± 4.8<br>G3: 77.3 ± 4.6<br>G4: 79.1 ± 5.9<br><br>Serum 25(OH)D (nmol/L)<br>G1: 54.8 ± 31.2<br>G2: 56.3 ± 10.7<br>G3: 52.5 ± 13.1<br>G4: 53.6 ± 12.4<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 30.3 ± 6.4<br>G2: 30.5 ± 5.3<br>G3: 30.7 ± 6.4<br>G4: 30.3 ± 6.2<br><br>Ethnicity (%)<br>White:<br>G1: 83.6<br>G2: 71.7<br>G3: 76.1<br>G4: 82.6<br>Black:<br>G1: 15.6<br>G2: 25.0<br>G3: 22.4<br>G4: 14.5 | Vitamin D <sub>3</sub><br><br>Doses<br>Dose-finding stage:<br>G1: vitamin D <sub>3</sub> 200 IU/d [5 µg/d]<br>G2: vitamin D <sub>3</sub> 1000 IU/d [25 µg/d]<br>G3: vitamin D <sub>3</sub> 2000 IU/d [50 µg/d]<br>G4: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d]<br><br>Best-dose stage: <sup>1,2)</sup><br>G1: vitamin D <sub>3</sub> 200 IU/d [5 µg/d]<br>G2–G4: vitamin D <sub>3</sub> 1000 IU/d [25 µg/d]<br><br><sup>2)</sup> Newly recruited participants and the participants in G3 and G4 were assigned vitamin D <sub>3</sub> 1000 IU/d [25 µg/d].<br><br>Background vitamin D intake (IU/d)<br>G1: 307 (647) IU/d [7.68 (16.2) µg/d]<br>G2: 293 (702) IU/d [7.33 (17.6) µg/d]<br>G3: 345 (779) IU/d [8.63 (19.5) µg/d]<br>G4: 237 (520) IU/d [5.93 (13.0) µg/d] | <b>Falls</b><br>- 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.<br>- 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<br>- when a fall was reported, staff called the participant to obtain additional information.<br><br><b>Serious falls</b><br>- defined as a fall resulting in a dislocated, broken, or | Data extracted: ITT<br><br><b>Dose-finding stage - Falls</b><br>First fall during dose-finding, <i>n</i> of participants<br>G1: 123<br>G2: 43<br>G3: 41<br>G4: 41<br><br>First fall during dose-finding, rate per 100 person-years (95% CI)<br>G1: 88.3 (74.0, 105.4)<br>G2: 70.5 (52.3, 95.0)<br>G3: 103.0 (75.8, 139.9)<br>G4: 99.4 (73.2, 135.0)<br><br>First fall during dose-finding, HR (95% CI)<br>G2 vs G1: 0.80 (0.57, 1.14)<br>G3 vs G1: 1.20 (0.84, 1.71)<br>G4 vs G1: 1.16 (0.82, 1.65)<br>G3 vs G2: 1.50 (0.98, 2.31)<br>G4 vs G2: 1.45 (0.94, 2.22)<br><br><b>Dose-finding stage - Serious falls</b><br>First serious fall during dose-finding, <i>n</i> of participants<br>G1: 12<br>G2: 4<br>G3: 5<br>G4: 6 | <b>2</b> |

| Reference Study<br>Country<br>Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|---|--|---|---|---|---|----------|
|   | <p>to agree to maintain this dosage.</p> <p>N participants, randomised/completed/analysed</p> <p>Dose-finding stage (Oct 2015 to Mar 2018):<br/>G1: 256/NR/NR<br/>G2: 121/118/121<br/>G3: 68/66/68<br/>G4: 69/66/69</p> <p>Best-dose stage<sup>1)</sup> (Apr 2018 to May 2019):<br/>G1: +38 = 339/329/339<br/>G2–G4: +91 = 349/338/ITT 349, PP 308</p> <p><sup>1)</sup> 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.</p> | <p>Other:<br/>G1: 2.0<br/>G2: 7.5<br/>G3: 3.0<br/>G4: 2.9</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> <p>Health status – Frailty (%)</p> <p>Robust:<br/>G1: 31.2<br/>G2: 25.8<br/>G3: 36.8<br/>G4: 26.1</p> <p>Pre-frail:<br/>G1: 57.8<br/>G2: 60.8<br/>G3: 52.9<br/>G4: 58.0</p> <p>Frail:<br/>G1: 10.9<br/>G2: 13.3<br/>G3: 10.3<br/>G4: 15.9</p> <p>Fallen at least once in prior year (%)<br/>G1: 63.3<br/>G2: 62.0<br/>G3: 63.2<br/>G4: 68.1</p> <p>Season: Oct 2015 to May 2019.</p> | <p>Background vitamin D supplement use (%)<br/>G1: 37.5<br/>G2: 39.7<br/>G3: 39.7<br/>G4: 33.3</p> <p>Background calcium intake: NR</p> <p><u>Compliance</u></p> <p>Pill count method (%)</p> <p>At least 80% of daily pills taken:<br/>G1: 75.8<br/>G2–G4: 72.5</p> <p>50%–79% of daily pills taken:<br/>G1: 13.9<br/>G2–G4: 11.5</p> <p>25%–49% of daily pills taken:<br/>G1: 4.4<br/>G2–G4: 8.0</p> <p>Less than 25% of daily pills taken:<br/>G1: 5.9<br/>G2–G4: 8.0</p> <p>Serum 25(OH)D (nmol/L) During dose-finding at 12 mo:<br/>G1: 68.3 ± 17.9<br/>G2: 81.0 ± 16.7<br/>G3: 83.3 ± 17.9<br/>G4: 123.2 ± 36.9</p> | <p>fractured body part, or a fall associated with a serious adverse event.</p> <p>****</p> <p>Assay of serum 25 (OH)D: HPLC–MS/MS</p> | <p>First fall with hospitalization during dose-finding, <i>n</i> of participants<br/>G1: 6<br/>G2: 2<br/>G3: 5<br/>G4: 1</p> <p>First serious fall during dose-finding, rate per 100 person-years (95% CI)<br/>G1: 5.4 (3.1, 9.5)<br/>G2: 4.9 (1.8, 13.1)<br/>G3: 7.3 (3.0, 17.5)<br/>G4: 8.9 (4.0, 19.9)</p> <p>First fall with hospitalization during dose-finding, rate per 100 person-years (95% CI)<br/>G1: 2.7 (1.2, 5.9)<br/>G2: 2.4 (0.6, 9.6)<br/>G3: 7.3 (3.0, 17.5)<br/>G4: 1.4 (0.2, 10.1)</p> <p>First serious fall during dose-finding, HR (95% CI)<br/>G2 vs G1: 0.91 (0.29, 2.81)<br/>G3 vs G1: 1.37 (0.48, 3.89)<br/>G4 vs G1: 1.68 (0.63, 4.48)</p> <p>First fall with hospitalization during dose-finding, HR (95% CI)<br/>G2 vs G1: 0.86 (0.17, 4.29)<br/>G3 vs G1: 2.84 (0.87, 9.30)<br/>G4 vs G1: 0.55 (0.07, 4.60)</p> |          |

| Reference Study<br>Country<br>Duration<br>Funding                 | Design                                | Subject characteristics at baseline <sup>(a)</sup> | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D                                | Results   | RoB Tier                            |
|---|---------------------------------------|--|---|---|---|-------------------------------------|
|   |                                       |  | During dose-finding + best dose stages at 24 mo:<br>G1: 70.4 ± 16.6<br>G2–G4: 99.2 ± 29.9 |   | <p><b>Dose-finding + best-dose stages - Falls</b><br/>First fall over 2 y, <i>n</i> of participants<br/>G1: 208<br/>G2–G4: 215</p> <p>First fall over 2 y, rate per 100 person-years (95% CI)<br/>G1: 74.8 (65.3, 85.7)<br/>G2–G4: 77.4 (67.8, 88.5)</p> <p>First fall over 2 y, HR (95% CI)<br/>G2–G4 vs G1: 1.05 (0.87, 1.27)</p> <p><u>Data extracted:</u> PP</p> <p><b>Dose-finding + best-dose stages- Falls</b><br/>First fall over 2 y, <i>n</i> of participants<br/>G1: 208<br/>G2–G4: 157</p> <p>First fall over 2 y, rate per 100 person-years (95% CI)<br/>G1: 74.8 (65.3, 85.7)<br/>G2–G4: 77.6 (66.3, 90.7)</p> <p>First fall over 2 y, HR (95% CI)<br/>G2–G4 vs G1: 0.96 (0.78, 1.18)</p> |                                     |
| Bischoff-Ferrari et al. (2020);<br>Bischoff-Ferrari et al. (2022) | RCT (parallel)<br>Inclusion criteria: | Sex (% females)<br>G1–G4: 61.4<br>G5–G8: 62.0      | Vitamin D <sub>3</sub><br>Doses   | <b>Fractures</b><br>- nonvertebral<br>- confirmed by x-ray reports or medical | <u>Data extracted:</u> unclear<br><b>Fractures</b>  | <b>1 for fractures; 2 for falls</b> |



| Reference<br>Study<br>Country<br>Duration<br>Funding  | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|---|--|--|---|---|---|----------|
| <p><b>DO-HEALTH</b></p> <p>Switzerland, Germany, Austria, France, and Portugal</p> <p>Latitude NA</p> <p>3 y</p> <p>Mixed funding</p> | <p>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.</p> <p>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.</p> <p>Exclusion criteria: Taking &gt;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</p> | <p>Age (y)</p> <p>G1–G4: 74.9 ± 4.4</p> <p>G5–G8: 75.0 ± 4.5</p> <p>Serum 25(OH)D (ng/mL)</p> <p>G1–G4: 22.4 ± 8.5 ng/mL [56 ± 21.25 nmol/L]</p> <p>G5–G8: 22.4 ± 8.4 ng/mL [56 ± 21 nmol/L]</p> <p>BMI (kg/m<sup>2</sup>)</p> <p>G1–G4: 26.2 ± 4.2</p> <p>G5–G8: 26.5 ± 4.4</p> <p>Ethnicity [assumed]: Majority Caucasian</p> <p>Smoking status: NR</p> <p>Alcohol use: NR</p> <p>Health status: Generally healthy</p> <p>Fallen in prior year (%)</p> <p>G1: 42.3</p> <p>G2: 41.4</p> <p>Season: NR</p> | <p>G1: double placebo + CEP</p> <p>G2: double placebo + SHEP</p> <p>G3: vitamin D placebo + omega-3 fatty acids 1 g/d + CEP</p> <p>G4: vitamin D placebo + omega-3 fatty acids 1 g/d + SHEP</p> <p>G5: vitamin D<sub>3</sub> 2000 IU/d [50 µg/d] + omega-3 fatty acid placebo + CEP</p> <p>G6: vitamin D<sub>3</sub> 2000 IU/d [50 µg/d] + omega-3 fatty acid placebo + SHEP</p> <p>G7: vitamin D<sub>3</sub> 2000 IU/d [50 µg/d] + omega-3 fatty acids 1 g/d + CEP</p> <p>G8: vitamin D<sub>3</sub> 2000 IU/d [50 µg/d] + omega-3 fatty acids 1 g/d + SHEP</p> <p>CEP = control exercise program;</p> <p>SHEP = simple home strength exercise program.</p> <p>Background vitamin D intake: NR</p> <p>Background vitamin D supplement use, ≥800 IU [20 µg] (%)</p> <p>G1–G4: 11.7</p> | <p>records describing an x-ray report or repair of the fracture.</p> <p><b>Falls</b></p> <p>- defined as unintentionally coming to rest on the ground, floor, or other lower level</p> <p>- 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</p> <p>- fall protocol collected information on the fall circumstances, related injuries, treatment, and related health-care utilization.</p> <p><b>Injurious falls</b></p> <p>- defined as falls leading to any injury.</p> <p>****</p> <p>Assay of serum 25 (OH)D: LC–MS</p> | <p>Fractures over 3 years, <i>n</i> of events</p> <p>G1–G4: 127</p> <p>G5–G8: 129</p> <p>Fractures, unadjusted IRR (99% CI)</p> <p>G5–G8 vs G1–G4: 1.02 (0.74, 1.40)</p> <p>Fractures, adjusted IRR (99% CI)<sup>3)</sup></p> <p>G5–G8 vs G1–G4: 1.03 (0.75, 1.43)</p> <p><u>Data extracted: ITT</u></p> <p><b>Falls</b></p> <p>Falls over 3 years, <i>n</i> of events</p> <p>G1–G4: 1673</p> <p>G5–G8: 1660</p> <p>≥1 fall over 3 years, <i>n</i> of participants</p> <p>G1–G4: 654</p> <p>G5–G8: 657</p> <p>Falls, unadjusted IRR (95% CI)</p> <p>G5–G8 vs G1–G4: 1.00 (0.90, 1.12)</p> <p>Falls, adjusted IRR (95% CI)<sup>4)</sup></p> <p>G5–G8 vs G1–G4: 1.03 (0.92, 1.14)</p> |          |

| Reference<br>Study<br>Country<br>Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup> | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>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<br>Background calcium intake: NR<br><u>Compliance</u><br>Pill count method – at least 80% of total study pills taken (%)<br>All: 85.8<br><br>Serum 25(OH)D at 3 y (ng/mL)<br>G1–G4: 24.4 ng/mL [61 nmol/L] on average<br>G5–G8: 37.6 ng/mL [94 nmol/L] on average. |  | <p>≥ 1 fall, unadjusted OR (95% CI)<br/>G5–G8 vs G1–G4: 1.02 (0.85, 1.22)</p> <p>≥ 1 fall, adjusted OR (95% CI)<sup>4)</sup><br/>G5–G8 vs G1–G4: 1.02 (0.85, 1.23).</p> <p><b>Injurious falls</b><br/>Injurious falls over 3 y, <i>n</i> of events<br/>G1–G4: 1068<br/>G5–G8: 1073</p> <p>≥1 injurious fall over 3 y, <i>n</i> of participants<br/>G1–G4: 548<br/>G5–G8: 570</p> <p>Injurious falls, unadjusted IRR (95% CI)<br/>G5–G8 vs G1–G4: 1.01 (0.90, 1.13)</p> <p>Injurious falls, adjusted IRR (95% CI)<sup>4)</sup><br/>G5–G8 vs G1–G4: 1.03 (0.92, 1.14).</p> <p>≥ 1 injurious fall, unadjusted OR (95% CI)<br/>G5–G8 vs G1–G4: 1.10 (0.92, 1.30)</p> <p>≥ 1 injurious fall, adjusted OR (95% CI)<sup>4)</sup></p> |          |

| Reference<br>Study<br>Country<br>Duration<br>Funding | Design  | Subject<br>characteristics at<br>baseline <sup>(a)</sup> | Intervention <sup>(a)</sup> | Endpoint assessed<br>Assay of serum<br>25(OH)D | Results  | RoB Tier |
|--|---|--|-----------------------------|--|--|----------|
|  | <p>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.<br/>Temporary exclusion: acute fracture in the last 6 weeks.</p> <p>N participants randomised/<br/>completed/analysed<br/>G1–G4: 1081/907/1081<br/>G5–G8: 1076/938/1076</p> |  |                             |  | <p>G5–G8 vs G1–G4: 1.11 (0.93, 1.33).<br/> <sup>3)</sup> adjusted for age, sex, prior falls, BMI, and study site.<br/> <sup>4)</sup> adjusted for study site, sex, age, previous fall, baseline BMI, and baseline use of walking aids.</p> |          |

| Reference<br>Study<br>Country<br>Duration<br>Funding   | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|--|---|---|--|---|---|----------|
| Burt et al. (2019)<br><br>Canada<br><br>Latitude [assumed]<br>51.05° N<br><br>3 y<br><br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>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).<br><br>Participants were requested to take no more than 200 IU [5 µg] per day of additional vitamin D (eg. a multivitamin supplement).<br>Exclusion criteria:<br>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)<br>G1: 42.9<br>G2: 48.5<br>G3: 47.5<br><br>Age (y)<br>G1: 62.2 ± 4.2<br>G2: 62.6 ± 4.3<br>G3: 61.9 ± 4.1<br><br>Years since menopause<br>G1: 12.6 ± 5.9<br>G2: 11.7 ± 7.3<br>G3: 12.5 ± 5.6<br>Serum 25(OH)D (nmol/L)<br>G1: 76.7 ± 21.0<br>G2: 81.3 ± 20.1<br>G3: 78.4 ± 18.4<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 27.7 ± 4.3<br>G2: 28.1 ± 5.0<br>G3: 27.1 ± 4.1<br><br>Ethnicity (%)<br>Non-Hispanic White:<br>G1: 93.3<br>G2: 96.9<br>G3: 97.0<br>Non-Hispanic black:<br>G1: 1.0<br>G2: 1.0<br>G3: 0<br>Asian: | Vitamin D <sub>3</sub><br><br>Doses:<br>G1: vitamin D <sub>3</sub> 400 IU/d [10 µg/d]<br>G2: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d]<br>G3: vitamin D <sub>3</sub> 10 000 IU/d [250 µg/d]<br><br>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.<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br>Pill count method – number of days of supplement administration vs total number of days (%)<br>All: 99 on average, range (range 81–100).<br><br>Serum 25(OH)D at 36 mo (nmol/L) | <b>Fractures</b><br>- reported as adverse events<br>- reviewed by study clinicians via participant medical records and x-ray reports where available<br>- fractures of fingers or toes and traumatic fractures were not included.<br><br>Low-trauma (fragility) fractures:<br>-reported as adverse events<br>- defined as resulting from low trauma, such as a fall from standing height or less.<br><br><b>Falls</b><br>- reported as adverse events<br>- adjudicated by the study physicians.<br><br>****<br><br>Assay of serum 25 (OH)D: CLIA (DiaSorin Liaison XL system) | Data extracted: unclear<br><br><b>Low-trauma fractures</b><br>Low-trauma fractures over 3 y, <i>n</i> of events<br>G1: 4<br>G2: 3<br>G3: 5<br><br>≥ 1 low-trauma fracture over 3 y, <i>n</i> of participants<br>G1: 4<br>G2: 3<br>G3: 5<br><br><b>Falls</b><br>Falls over 3 y, <i>n</i> of events<br>G1: 4<br>G2: 11<br>G3: 6<br><br>≥ 1 fall over 3 y, <i>n</i> of participants<br>G1: 4<br>G2: 10<br>G3: 5<br><br>Stated that no significant difference in falls detected among the 3 groups. | <b>1</b> |

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

| Reference<br>Study<br>Country<br>Duration<br>Funding              | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D   | Results  | RoB Tier |
|---|---|---|---|--|--|----------|
| [study residents in three states]<br><br>2 y<br><br>Mixed funding | 25(OH)D level > 25 nmol/L and < 90 nmol/L.<br><br>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.<br><br>N participants randomised/completed/analysed | Serum 25(OH)D (%)<br>25–40 nmol/L:<br>G1: 54<br>G2: 61<br>41–60 nmol/L:<br>G1: 35<br>G2: 28<br>61–90 nmol/L:<br>G1: 11<br>G2: 11<br><br>BMI: NR<br><br>Ethnicity [assumed]: Majority Caucasian<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: NR<br>Previous fracture (%)<br>Hip:<br>G1: 12<br>G2: 17<br>Colles:<br>G1: 5<br>G2: 3<br>Recorded vertebral:<br>G1: 6<br>G2: 4<br>Other:<br>G1: 7<br>G2: 9<br>Any:<br>G1: 24<br>G2: 27 | wk = 35.71 µg/d] until Nov 1998; thereafter 1000 IU/d [=25 µg/d] <sup>6)</sup> + calcium 600 mg/d.<br><br><sup>6)</sup> due to the discontinuation of the preparation of commercial 10 000 IU [250 µg] vitamin D <sub>2</sub> tablets.<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br>Background calcium supplement use (%)<br>G1: 5<br>G2: 4<br><br><u>Compliance</u><br><br>Pill count method - vitamin D supplements removed from the participant's medication container (%)<br>0–25%:<br>G1: 5<br>G2: 4<br>26–50%:<br>G1: 7<br>G2: 7<br>51–75%:<br>G1: 21 | person coming to rest inadvertently on the ground or other lower level<br>- recorded by residential care staff prospectively in diaries.<br><br>*****<br><br>Assay of serum 25 (OH)D: NR | ≥ 1 fracture, OR (95% CI)<br>G2 vs G1: 0.69 (0.40, 1.18)<br><br><b>Falls</b><br>Falls over 2 y, <i>n</i> of events<br>G1: 890<br>G2: 665<br><br>≥ 1 fall over 2 y, <i>n</i> of participants<br>G1: 185<br>G2: 170<br><br>Falls, IRR (95% CI)<br>G2 vs G1: 0.73 (0.57, 0.95)<br><br>≥ 1 fall, OR (95% CI)<br>G2 vs G1: 0.82 (0.59, 1.12)<br><br><u>Data extracted: PP<sup>5)</sup></u><br><br><b>Fractures</b><br>≥ 1 fracture over 2 y, <i>n</i> of participants<br>G1: 30<br>G2: 21<br><br>≥ 1 fracture, OR (95% CI)<br>G2 vs G1: 0.68 (0.38, 1.22)<br><br><b>Falls</b><br>Falls over 2 y, <i>n</i> of events<br>G1: 862<br>G2: 570<br><br>≥ 1 fall over 2 y, <i>n</i> of participants<br>G1: 176 |          |



| Reference<br>Study<br>Country<br>Duration<br>Funding  | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results  | RoB Tier |
|---|---|---|--|---|--|----------|
|   | G1: 312/unclear/ITT 312, PP 271 <sup>5)</sup><br>G2: 313/unclear/ITT 313, PP 269 <sup>5)</sup><br><br><sup>5)</sup> those whose vitamin D compliance was $\geq 50\%$  | Season: NR  | G2: 18<br>76–100%:<br>G1: 66<br>G2: 68<br>Unknown:<br>G1: 1<br>G2: 3<br><br>Serum 25(OH)D at 2 yr: NR  |   | G2: 152<br><br>Falls, IRR (95% CI)<br>G2 vs G1: 0.63 (0.48, 0.82)<br><br>$\geq 1$ fall, OR (95% CI)<br>G2 vs G1: 0.70 (0.50, 0.99)<br><br><sup>5)</sup> those whose vitamin D compliance was $\geq 50\%$   |          |
| Grant et al. (2005)<br><br><b>RECORD</b><br><br>United Kingdom<br><br>Latitude [assumed] 51° N to 58° N; the trial was based in 21 hospitals in United Kingdom<br><br>24 to 62 mo (median 45, IQR 37–52)<br><br>Mixed funding | RCT (parallel)<br><br>Inclusion criteria:<br>People aged $\geq 70$ years who had had a low trauma, osteoporotic fracture in the previous 10 years.<br><br>Exclusion criteria:<br>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)<br>G1–G2: 85<br>G3–G4: 85<br><br>Age (y)<br>G1–G2: $77 \pm 6$<br>G3–G4: $77 \pm 6$<br><br>Serum 25(OH)D (ng/mL)<br>All: $15.2 \pm 6.5$ ng/mL [ $38 \pm 16.25$ nmol/L]<br>NR across groups.<br><br>BMI: NR<br><br>Ethnicity (%)<br>White:<br>G1–G2: 99<br>G3–G4: 99<br><br>Smoking status (%)<br>Current smokers:<br>G1–G2: 12.1 | Vitamin D <sub>3</sub><br><br>Doses<br>G1: double placebo<br>G2: vitamin D placebo + calcium 1000 mg/d<br>G3: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + calcium placebo<br>G4: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + calcium 1000 mg/d<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br>Pill count method – taking tablets $> 80\%$ of days at 24 mo (%): | <b>Fractures</b><br>- clinical, radiological confirmed vertebral fractures excluding those of the face or skull<br>- 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 | <u>Data extracted:</u> ITT<br><br><b>New fractures</b><br>New fractures over the intervention, <i>n</i> of participants<br>G1–G2: 385<br>G3–G4: 396<br><br>New fractures, HR (95% CI)<br>G3–G4 vs G1–G2: 1.01 (0.88, 1.17)<br><br><b>Confirmed fractures</b><br>Confirmed fractures over the intervention, <i>n</i> of participants<br>G1–G2: 377<br>G3–G4: 387<br><br>Confirmed fractures, HR (95% CI)<br>G3–G4 vs G1–G2: 1.01 (0.87, 1.16) | <b>2</b> |

| Reference<br>Study<br>Country<br>Duration<br>Funding  | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results  | RoB Tier |
|---|--|---|---|---|--|----------|
|   | <p>to be leaving the UK; daily intake of &gt;200 IU [5 µg/d] vitamin D or &gt; 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.</p> <p>N participants randomised/completed/analysed<br/>G1–G2: 2643/2382 at 24 mo, 572 at 48 mo, 29 at 60 mo<br/>/2382 at 24 mo, 572 at 48 mo, 29 at 60 mo<br/>G3–G4: 2649/2401 at 24 mo, 582 at 48 mo, 24 at 60 mo<br/>/2401 at 24 mo, 582 at 48 mo, 24 at 60 mo</p> | <p>G3–G4: 11.3</p> <p>Alcohol use: NR</p> <p>Health status: A history of low-trauma, osteoporotic fracture in the previous 10 years.</p> <p>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.</p> <p>Previous fracture since age 50 y (%)<br/>G1: 35.3<br/>G2: 35.0</p> <p>Season: Feb 1999 to March 2002.</p> | <p>G1–G2: 78.8<br/>G3–G4: 78.4</p> <p>Completing questionnaires &gt;80% of days at 24 mo (%):<br/>G1–G2: 59.9<br/>G3–G4: 60.6</p> <p>Serum 25(OH)D, change over 1 yr (ng/mL)<br/>G1: +3.1 ± 7.2 ng/mL [7.75 ± 18 nmol/L]<br/>G2: +1.4 ± 5.7 ng/mL [3.5 ± 14.25 nmol/L]<br/>G3: +9.7 ± 8.7 ng/mL [24.25 ± 21.75 nmol/L]<br/>G4: +9.6 ± 6.9 ng/mL [24.0 ± 17.25 nmol/L]</p> | <p>Hospital Episode Statistics gathered by the Department of Health in England</p> <p>- confirmation always sought from a second source.</p> <p><b>Falls</b></p> <p>- defined by means of the question "Have you fallen during the last week?"</p> <p>- obtained from the postal questionnaires and were supplemented by further clinical information on potentially serious adverse events.</p> <p>****</p> <p>Assay of serum 25 (OH)D: HPLC</p> | <p><b>Low-trauma fractures</b></p> <p>Low-trauma fractures over the intervention, <i>n</i> of participants<br/>G1–G2: 345<br/>G3–G4: 353</p> <p>Low-trauma fractures, HR (95% CI)<br/>G3–G4 vs G1–G2: 1.02 (0.88, 1.19)</p> <p><b>Falls</b></p> <p>Falls over the intervention, <i>n</i> of participants<br/>G1–G2: 381<br/>G3–G4: 380</p> <p>Falls, HR (95% CI)<br/>G3–G4 vs G1–G2: 0.97 (0.84, 1.12)</p> |          |
| <p>Hin et al. (2016)</p> <p><b>BEST-D</b></p> <p>United Kingdom</p> <p>Latitude [assumed] 51° N to 58° N</p> <p>12 mo</p> | <p>RCT (parallel)</p> <p>Inclusion criteria: Ambulatory, aged 65 y or more, living in the community, not currently taking more than 10 µg/d vitamin D<sub>3</sub>.</p>   | <p>Sex (% females)<br/>G1: 49<br/>G2: 50<br/>G3: 49</p> <p>Age (y)<br/>G1: 72 ± 6<br/>G2: 72 ± 6</p>  | <p>Vitamin D<sub>3</sub></p> <p>Doses<br/>G1: placebo<br/>G2: vitamin D<sub>3</sub> 2000 IU/d [50 µg/d]<br/>G3: vitamin D<sub>3</sub> 4000 IU/d [100 µg/d]</p>  | <p><b>Fractures</b></p> <p>- self-reported</p> <p>- measured at all sites and specific sites</p> <p>- recorded at 6 and 12 mo</p> <p>- no additional information provided.</p>  | <p><u>Data extracted:</u> ITT</p> <p><b>Any fracture</b>, <i>n</i> of participants<br/>G1: 1<br/>G2–G3: 6<br/><i>P</i> = 0.31 for difference in numbers between groups.</p>  | <b>2</b> |

| Reference<br>Study<br>Country<br>Duration<br>Funding | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|--|---|--|---|---|---|----------|
| Mixed funding  | <p>Exclusion criteria:<br/>Nursing home residents;<br/>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).</p> <p>N participants randomised/completed/analysed<br/>G1: 101/95/101<br/>G2: 102/98/102<br/>G3: 102/97/102</p> | <p>G3: 71 ± 6</p> <p>Serum 25(OH)D (nmol/L)<br/>G1: 47 ± 1.5<br/>G2: 55 ± 2.2<br/>G3: 49 ± 1.5</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 28 ± 5<br/>G2: 27 ± 4<br/>G3: 27 ± 5</p> <p>Ethnicity: White</p> <p>Smoking status (%)<br/>Current smokers:<br/>G1: 7<br/>G2: 7<br/>G3: 7</p> <p>Alcohol use: NR</p> <p>Health status (%)<br/>Hypertension:<br/>G1: 35<br/>G2: 43<br/>G3: 39</p> <p>Diabetes:<br/>G1: 9<br/>G2: 9<br/>G3: 9</p> <p>Fracture experienced ever (%)<br/>G1: 30<br/>G2: 29<br/>G3: 30</p> | <p>Background vitamin D intake: NR</p> <p>Background vitamin D supplement use ≤10 µg/d (%)<br/>G1: 13<br/>G2: 10<br/>G3: 12</p> <p>Background calcium intake (mg/d)<br/>G1: 713 ± 302<br/>G2: 695 ± 292<br/>G3: 724 ± 287</p> <p>Background calcium supplement use (%)<br/>G1: 4<br/>G2: 1<br/>G3: 4</p> <p><u>Compliance</u><br/>Pill count method – capsules taken on all or most days (%)<br/>At 6 mo:<br/>G1: 87<br/>G2: 93<br/>G3: 93<br/>At 12 mo:<br/>G1: 85<br/>G2: 92<br/>G3: 90</p> | <p><b>Falls</b><br/>- self-reported<br/>- recorded at 6 and 12 mo<br/>- no additional information provided.</p> <p>****</p> <p>Assay of serum 25 (OH)D: CLIA (Beckman Coulter Ltd., High Wycombe, England).</p> | <p><b>Any fall</b>, <i>n</i> of participants<br/>G1: 14<br/>G2–G3: 34<br/><i>P</i> = 0.53 for difference in numbers between groups.</p> |          |

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

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

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



| Reference<br>Study<br>Country<br>Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results  | RoB Tier |
|--|--|---|--|---|--|----------|
|  | <p>systolic or &gt; 99 mmHg diastolic); not taking medication (hypotensive, hypolipemic, anti-inflammatory, oral corticosteroid) likely to affect vitamin D metabolism or cardiovascular disease risk.</p> <p>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.</p> <p>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.</p> <p>N participants randomised/ completed/analysed<br/>G1: 102/90/ITT 90, PP 88<br/>G2: 102/85/ITT 84, PP 84<br/>G3: 101/90/ITT 90, PP 88</p> | <p>G3: 33.2 ± 13.8</p> <p>BMI (kg/m<sup>2</sup>)<br/>G1: 25.9 ± 3.8<br/>G2: 25.3 ± 3.9<br/>G3: 25.2 ± 3.4</p> <p>Ethnicity: White</p> <p>Smoking status: Non-smokers</p> <p>Alcohol use: NR</p> <p>Health status: Healthy</p> <p>Season: Jan–March at baseline.</p> | <p>G3: 832 IU [20.8 µg/d]</p> <p>Background vitamin D intake (µg/d)<br/>G1: 5.6 ± 3.0<br/>G2: 4.6 ± 2.5<br/>G3: 5.3 ± 2.9</p> <p>Background calcium intake (mg/d)<br/>G1: 1291 ± 492<br/>G2: 1261 ± 488<br/>G3: 1306 ± 568</p> <p><u>Compliance</u><br/>Pill count method (%)<br/>All: 92 on average (range 72–98)</p> <p>Serum 25(OH)D at 1 yr (nmol/L)<br/>G1: 32.0 ± 1.6<br/>G2: 65.0 ± 2.1<br/>G3: 76.0 ± 2.0 reported as mean ± SEM</p> |   | <p>woman having two fractures on two separate occasions:<br/>- wrist/lower arm, <i>n</i> = 3<br/>- foot/ankle, <i>n</i> = 3<br/>- clavicle, <i>n</i> = 1</p> |          |
| <p>Peacock et al. (2000)<br/>USA, Indiana</p>        | <p>RCT (parallel)</p> <p>Inclusion criteria: Women and men aged 60 and over.</p>   | <p>Sex (% females)<br/>G1: 73<br/>G2: 71<br/>G3: 72</p>   | <p>Calcidiol</p> <p>Doses [labeled]<br/>G1: placebo<br/>G2: calcium 750 mg/d</p>   | <p><b>Fractures</b><br/>- vertebral fractures: defined as a reduction of the anterior</p> | <p><u>Data extracted:</u> unclear</p> <p><b>Fractures</b><br/>Nonvertebral fractures over 4 y, <i>n</i> of events</p>  | <b>1</b> |

| Reference Study Country Duration Funding             | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed Assay of serum 25(OH)D   | Results   | RoB Tier |
|--|--|---|---|--|---|----------|
| Latitude [assumed] 42.74° N<br>4 y<br>Public funding | <p>Exclusion criteria:<br/>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.</p> <p>N participants<br/>Randomised:<br/>All: 437<br/>Completed:<br/>All: 236<br/>Baseline information available<sup>7)</sup>:<br/>Females<br/>G1: 98<br/>G2: 89<br/>G3: 95<br/>Male<br/>G1: 37<br/>G2: 37<br/>G3: 37</p> <p><sup>7)</sup> 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.</p> | <p>Age (y)<br/>Females<br/>G1: 72.3 ± 7.5<br/>G2: 73.9 ± 8.0<br/>G3: 74.1 ± 8.3<br/>Male<br/>G1: 75.4 ± 7.6<br/>G2: 76.0 ± 7.7<br/>G3: 75.5 ± 7.2<br/>Serum 25(OH)D (nmol/L)<br/>Females<br/>G1: 60.0 ± 30<br/>G2: 62.5 ± 25<br/>G3: 57.5 ± 33<br/>Male<br/>G1: 65.0 ± 30<br/>G2: 67.5 ± 23<br/>G3: 65.0 ± 25</p> <p>BMI: NR<br/>Ethnicity: White<br/>Smoking status: NR<br/>Alcohol use: NR<br/>Health status: NR<br/>Season: NR</p> | <p>G3: calcidiol 15 µg/d</p> <p>Analysed doses:<br/>Capsules analysed every 6 mo; no significant decrease observed over 4 y.</p> <p>Background vitamin D intake: NR</p> <p>Background calcium intake (mg/d)<br/>Females<br/>G1: 586 ± 290<br/>G2: 564 ± 294<br/>G3: 572 ± 267<br/>Male<br/>G1: 629 ± 249<br/>G2: 670 ± 325<br/>G3: 739 ± 335</p> <p><u>Compliance</u><br/>Pill count method – calcidiol capsules at each visit (%)<br/>All: 89 ± 16<br/>Pill count method – calcium tablets at each visit (%)<br/>All: 80 ± 20<br/>Pill count method – placebo capsules at each visit (%):<br/>All: 85 ± 19<br/>Serum 25(OH)D at the endpoint: NR</p> | <p>vertebral height of 20% or greater<br/>- obtained from diaries kept by the participants<br/>- 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.<br/>- 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.</p> <p>****<br/>Assay of serum 25 (OH)D: CPBA (vitamin D-binding protein from rat serum) after HPLC</p> | <p>Females<br/>G1: 9<br/>G2: 9<br/>G3: 10<br/>Male<br/>G1: 1<br/>G2: 2<br/>G3: 4</p> <p>Vertebral fractures over 4 y, <i>n</i> of events<br/>Females<br/>G1: 10<br/>G2: 5<br/>G3: 15<br/>Male<br/>G1: 3<br/>G2: 2<br/>G3: 4</p> <p>Total fractures over 4 y, <i>n</i> of events<br/>Females<br/>G1: 19<br/>G2: 14<br/>G3: 25<br/>Male<br/>G1: 4<br/>G2: 4<br/>G3: 8</p> |          |

| Reference<br>Study<br>Country<br>Duration<br>Funding                          | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|---|---|--|--|---|---|----------|
| Prince et al. (2008)<br>Australia<br>Latitude 32° S<br>1 yr<br>Public funding | RCT (parallel)<br><br>Inclusion criteria:<br>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].<br><br>Exclusion criteria:<br>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<br><br>Age (y)<br>G1: $77.4 \pm 5.0$<br>G2: $77.0 \pm 4.2$<br>Serum 25(OH)D (ng/mL)<br>G1: $17.7 \pm 5.1$ ng/mL [ $44.3 \pm 12.8$ nmol/L]<br>G2: $18.1 \pm 5.0$ ng/mL [ $45.3 \pm 12.5$ nmol/L]<br><br>BMI: NR<br><br>Ethnicity: NR<br><br>Smoking status: NR<br><br>Alcohol use: NR<br><br>Health status: NR<br><br>Number of falls in the past 12 mo (%)<br>1:<br>G1: 57.6<br>G2: 59.6<br>2:<br>G1: 26.5<br>G2: 27.2<br>3:<br>G1: 13.2<br>G2: 9.9<br>> 3:<br>G1: 2.6<br>G2: 3.3 | Vitamin D <sub>2</sub><br>Doses<br>G1: placebo + calcium 1000 mg/d<br>G2: vitamin D <sub>2</sub> 1000 IU/d [25 µg/d] + calcium 1000 mg/d.<br><br>Background vitamin D intake: NR<br><br>Background calcium intake (mg/d)<br>G1: $1080 \pm 426$<br>G2: $1067 \pm 484$<br><br><u>Compliance</u><br>Pill count method (%)<br>G1: 86<br>G2: 86<br><br>Serum 25(OH)D in winter/spring<br>G2 vs. G1: 28.1% higher.<br><br>Serum 25(OH)D in summer/autumn<br>G2 vs. G1: 12.5% higher. | <b>Fractures</b><br>- reported as adverse events.<br><b>Falls</b><br>- defined as unintentionally coming to rest on the ground, floor, or other lower level<br>- 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.<br><br>*****<br>Assay of serum 25 (OH)D: RIA (Dia-Sorin, Stillwater, Minnesota) | Data extracted: ITT<br><br><b>Fractures</b><br>Fractures over 1 yr, rate (%)<br>G1: 2.0<br>G2: 2.6<br><br><b>Falls</b><br>$\geq 1$ fall, <i>n</i> of participants<br>G1: 95<br>G2: 80<br><br>$1$ fall, OR (95% CI)<br>G2 vs G1: 0.50 (0.28, 0.88)<br><br>$\geq 1$ fall, OR (95% CI)<br>G2 vs G1: 0.66 (0.41, 1.06)<br><br>$\geq 1$ fall adjusted for baseline height, OR (95% CI)<br>G2 vs G1: 0.61 (0.37, 0.99)<br><br>$\geq 2$ falls, OR (95% CI)<br>G2 vs G1: 0.86 (0.50, 1.49)<br><br>First fall in summer/autumn, OR (95% CI)<br>G2 vs G1: 0.81 (0.46, 1.42)<br><br>First fall in winter/spring, OR (95% CI)<br>G2 vs G1: 0.55 (0.32, 0.96)<br>First fall in winter/spring, RR (95% CI)<br>G2 vs G1: 0.77 (0.56, 0.98) | <b>2</b> |

| Reference<br>Study<br>Country<br>Duration<br>Funding  | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results   | RoB Tier |
|---|--|--|--|---|---|----------|
| Smith et al. (2017)<br><br>(Earlier publication by Gallagher et al. 2012 was used for baseline information)<br><b>VIDOS</b><br>USA<br>Latitude 41° N<br>1 yr<br>Mixed funding | N participants randomised/completed/analysed<br>G1: 151/131/ ITT 151<br>G2: 151/124/ ITT 151<br><br>RCT (parallel)<br><br>Inclusion criteria:<br>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<br><br>Exclusion criteria:<br>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 | Season: Recruitment from Apr 2003 to Oct 2004.<br><br>Sex: Females<br><br>Age (y)<br>G1: 66 ± 6.5<br>G2: 68 ± 8.6<br>G3: 68 ± 8.1<br>G4: 66 ± 7.4<br>G5: 66 ± 6.3<br>G6: 69 ± 7.7<br>G7: 66 ± 7.1<br>G8: 65 ± 6.1<br>Serum 25(OH)D (nmol/L)<br>G1: 37.7 ± 9.1<br>G2: 37.8 ± 10.8<br>G3: 39.0 ± 9.5<br>G4: 37.4 ± 10.2<br>G5: 38.2 ± 10.1<br>G6: 39.8 ± 8.2<br>G7: 37.2 ± 9.2<br>G8: 38.6 ± 9.1<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 31.1 ± 5.2<br>G2: 30.3 ± 5.4<br>G3: 28.2 ± 6.1<br>G4: 30.0 ± 5.4<br>G5: 30.4 ± 5.4<br>G6: 30.2 ± 5.7<br>G7: 29.7 ± 6.4<br>G8: 32.1 ± 6.2 | Vitamin D <sub>3</sub><br><br>Doses<br>G1: placebo + calcium<br>G2: vitamin D <sub>3</sub> 400 IU/d [10 µg/d] + calcium<br>G3: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + calcium<br>G4: vitamin D <sub>3</sub> 1600 IU/d [40 µg/d] + calcium<br>G5: vitamin D <sub>3</sub> 2400 IU/d [60 µg/d] + calcium<br>G6: vitamin D <sub>3</sub> 3200 IU/d [80 µg/d] + calcium<br>G7: vitamin D <sub>3</sub> 4000 IU/d [100 µg/d] + calcium<br>G8: vitamin D <sub>3</sub> 4800 IU/d [120 µg/d] + calcium<br><br>Calcium to maintain total intake between 1200 to 1400 mg/d (was based on a baseline 7-day food diary)<br><br>Background vitamin D intake (IU/d)<br>G1: 105 ± 61 IU/d [2.6 ± 1.5 µg/d]<br>G2: 98 ± 58 IU/d [2.5 ± 1.5 µg/d] | <b>Falls</b><br>- 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<br>- details on the number of falls and whether a fall resulted in a break or fracture were collected by an interviewer every 3 mo.<br><br>****<br><br>Assay of serum 25 (OH)D: RIA (Diasorin kit) and LC-MS | <b>Data extracted:</b> unclear<br><br>≥ 1 fall over 1 yr, rate (%)<br>G1: 58<br>G2-G3: 53<br>G4-G6: 30<br>G7-G8: 69<br>P = 0.030 for difference in rates between groups.<br>Lower faller rate in G4-G6 vs the other groups, P < 0.05.<br><br>Falls over 1 yr, mean ± SE <sup>8)</sup><br>G1: 0.94 ± 0.23<br>G2-G3: 0.85 ± 0.19<br>G4-G6: 0.41 ± 0.09<br>G7-G8: 0.79 ± 0.19<br>P = 0.030 for difference between groups.<br>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.<br>≥ 1 fall, OR (95% CI) <sup>9)</sup><br>G1 vs G4-G6: 3.86 (1.24, 12.04) | <b>2</b> |

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

| Reference<br>Study<br>Country<br>Duration<br>Funding                                     | Design   | Subject<br>characteristics at<br>baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D  | Results  | RoB Tier |
|--|--|---|---|---|--|----------|
|  | publication the study referred to (Gallagher et al. 2012)]   | G5: 52<br>G6: 70<br>G7: 70<br>G8: 80<br><br>Health status:<br>Healthy.<br><br>Fallen in the previous 12 mo (%)<br>Caucasian: 32.2<br>African<br>American: 25.3<br><br>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)<br>Finland<br>Latitude [assumed] 61.5° N<br>2 y<br>Mixed funding | RCT (parallel)<br><br>Inclusion criteria:<br>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.<br><br>Exclusion criteria:<br>Participating in moderate to vigorous exercise >2 h/wk; regular use of vitamin D or | Sex: Females<br><br>Age (y)<br>G1: 73.8 ± 3.1<br>G2: 74.8 ± 2.9<br>G3: 74.1 ± 3.0<br>G4: 74.1 ± 2.9<br><br>Serum 25(OH)D (ng/mL)<br>G1: 27.1 ± 7.5 ng/mL [67.8 ± 18.8 nmol/L]<br>G2: 27.8 ± 7.2 ng/mL [69.5 ± 18.0 nmol/L]                                      | Vitamin D <sub>3</sub><br><br>Doses<br>G1: placebo + no exercise<br>G2: placebo + exercise<br>G3: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + no exercise<br>G4: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + exercise<br><br>Exercise = supervised, progressive group training classes 2 times/wk for the | <b>Falls</b><br>- defined as an unexpected event in which the participant comes to rest on the ground, floor or lower level<br>- obtained from prospective diaries that were returned monthly via mail; details of each fall ascertained by a phone call. | <u>Data extracted:</u> ITT<br><br><b>Falls</b><br>All falls, rate per 100 Person-years<br>G1: 118.2<br>G2: 120.7<br>G3: 132.1<br>G4: 113.1<br><br>All falls, IRR (95% CI)<br>G2 vs G1: 1.07 (0.77, 1.45)<br>G3 vs G1: 1.08 (0.78, 1.52)<br>G4 vs G1: 0.99 (0.72, 1.39)<br><br>Fallers, HR (95% CI) | <b>1</b> |



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

| Reference<br>Study<br>Country<br>Duration<br>Funding                            | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed<br>Assay of serum<br>25(OH)D   | Results  | RoB Tier |
|---|---|---|---|--|--|----------|
|   |   | Osteoarthritis:<br>G1: 23<br>G2: 25<br>G3: 28<br>G4: 33<br>Depression:<br>G1: 2.0<br>G2: 1.9<br>G3: 4.9<br>G4: 4.9<br>Season: April 2010 to March 2013.   |   |  |  |          |
| Wood et al. (2014)<br>United Kingdom<br>Latitude 57° N<br>1 yr<br>Mixed funding | RCT (parallel)<br>Inclusion criteria:<br>Healthy postmenopausal women aged 60–70 years, BMI 18–45 kg/m <sup>2</sup> .<br>Exclusion criteria:<br>Severe disease; taking vascular medications or dietary supplements known to contain vitamin D <sub>2</sub> or D <sub>3</sub> ; current smokers; participants with abnormal blood biochemistry at study screening.<br>N participants randomised/completed/analysed<br>G1: 102/91/100<br>G2: 102/84/97<br>G3: 101/90/96 | Sex: Females<br>Age (y)<br>G1: 63.9 ± 2.3<br>G2: 63.5 ± 1.9<br>G3: 64.1 ± 2.3<br>Serum 25(OH)D (nmol/L)<br>G1: 36.18 ± 17.1<br>G2: 32.74 ± 12.9<br>G3: 32.41 ± 13.8<br>BMI<br>G1: 26.6 ± 4.4<br>G2: 26.6 ± 4.2<br>G3: 26.8 ± 4.2<br>Ethnicity: Caucasian<br>Smoking status: Non-smokers.<br>Alcohol use: NR | Vitamin D <sub>3</sub><br>Doses [labeled]<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 400 IU/d [10 µg/d]<br>G3: vitamin D <sub>3</sub> 1000 IU/d [25 µg/d]<br>Analysed doses<br>G2: 346 IU [8.65 µg]<br>G3: 832 IU [20.8 µg]<br>Background vitamin D intake: NR<br>Background calcium intake: NR<br>Compliance<br>Pill count method (%)<br>All: >95%<br>Serum 25(OH)D, change over 12 mo (nmol/L), | Falls<br>- not defined<br>- recorded at each study visit by asking the question “Have you had any recent falls in the past 2 months?”<br>*****<br>Assay of serum 25 (OH)D: MS/MS | Data extracted: ITT<br>Falls over 12 mo, <i>n</i> of events<br>G1: 40<br>G2: 48<br>G3: 30<br>≥1 fall over 12 mo, <i>n</i> of participants<br>G1: 31<br>G2: 33<br>G3: 27<br><i>P</i> = 0.65 for difference in incidence between groups. | <b>2</b> |

| Reference<br>Study<br>Country<br>Duration<br>Funding | Design | Subject<br>characteristics at<br>baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed<br>Assay of serum<br>25(OH)D | Results | RoB Tier |
|--|--------|--|--|--|---------|----------|
|  |        | Health status:<br>Relatively healthy.<br><br>Fallen over the last 2<br>mo prior to the<br>study (%)<br>G1: 7<br>G2: 8<br>G3: 8<br><br>Season: Jan–March<br>2009 at baseline. | mean (95% CI)<br>G1: -2.72 (-5.15,<br>-0.29)<br>G2: +33.04 (29.03,<br>37.06)<br>G3: +42.90 (39.09,<br>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<sub>3</sub> - 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<sup>th</sup>–75<sup>th</sup> percentiles or the length between the percentiles.

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

| Reference Study<br>Country<br>Duration<br>Funding   | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>  | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D   | Results <sup>(a)</sup>  | RoB Tier                              |
|---|--|---|--|--|---|---------------------------------------|
| <b>Children and adolescents</b>   |  |   |  |  |   |                                       |
| Al-Shaar et al. (2013)<br>Lebanon<br>Latitude [assumed] 33.9° N<br>1 yr<br>Private funding<br>Based on the same study as El-Hajj Fuleihan et al. (2006) below but reported different outcomes and groups. | RCT (parallel)<br><br>Inclusion criteria:<br>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.<br><br>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.<br><br>N participants randomised/completed/analysed<br>Females:<br>G1: 58/55/55<br>G2: 62/58/58<br>G3: 59/55/54<br>Males:<br>G1: 61/56/56 | Sex (% females)<br>All: 49.3<br><br>Age (y)<br>Females:<br>G1: 13.60 (11.65–15.65)<br>G2: 12.56 (11.07–14.75)<br>G3: 13.08 (11.13–14.72)<br>Males:<br>G1: 12.9 (11.8–14.8)<br>G2: 12.6 (11.2–14.6)<br>G3: 12.9 (11.4–14.8)<br><br>Serum 25(OH)D (ng/mL)<br>Females:<br>G1: 11.7 (8.7–11.7) ng/mL [29.3 (21.8–29.3) nmol/L]<br>G2: 11.3 (8.7–15.6) ng/mL [28.3 (21.8–39.0) nmol/L]<br>G3: 12.6 (10.0–18.7) ng/mL [31.5 (25.0–46.8) nmol/L]<br>Males:<br>G1: 16.0 (12.9–19.5) ng/mL [40.0 (32.3–48.8) nmol/L] | Vitamin D <sub>3</sub><br><br>Doses<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 1400 IU/wk [35 µg/wk = 5 µg/d]<br>G3: vitamin D <sub>3</sub> 14 000 IU/wk [350 µg/wk = 50 µg/d]<br><br>Analysed doses:<br>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.<br><br>Background vitamin D intake: NR<br><br>Background calcium intake (mg/d)<br>Reported in the earlier publication on girls (El-Hajj Fuleihan, 2006)<br>G1: 672 ± 323<br>G2: 674 ± 364<br>G3: 686 ± 411 | BMD; DXA.<br><br>Also measured in hip structural analysis: Cross sectional area, outer diameter, section modulus, and buckling ratio.<br><br>****<br><b>BMD at baseline (g/cm<sup>2</sup>)</b><br><b>Females</b><br>Narrow neck:<br>G1: 0.99 ± 0.2<br>G2: 0.99 ± 0.2<br>G3: 0.97 ± 0.2<br><br>Shaft:<br>G1: 1.38 ± 0.24<br>G2: 1.34 ± 0.24<br>G3: 1.35 ± 0.24<br><br>Intertrochanteric:<br>G1: 0.94 ± 0.20<br>G2: 0.91 ± 0.15<br>G3: 0.89 ± 0.16 | Data extracted: unclear<br><br><b>BMD, change over 1 yr (%), mean ± SE</b><br><b>Females</b><br>Narrow neck:<br>G1: 5.25 ± 0.96<br>G2: 7.50 ± 0.92<br>G3: 6.12 ± 0.98<br><i>P</i> = 0.72 for difference in change between groups. <sup>4)</sup><br><br>Shaft:<br>G1: 8.32 ± 0.84<br>G2: 9.10 ± 0.85<br>G3: 8.15 ± 0.84<br><i>P</i> = 0.70 for difference in change between groups. <sup>4)</sup><br><br>Intertrochanteric:<br>G1: 5.18 ± 0.91<br>G2: 8.10 ± 0.88<br>G3: 5.84 ± 0.92<br><i>P</i> = 0.06 for difference in change between groups. <sup>4)</sup><br><br><sup>4)</sup> adjusted for baseline height, percentage change in lean mass and height, sun | <b>1 for females;<br/>2 for males</b> |

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

| Reference Study<br>Country<br>Duration<br>Funding  | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D                    | Results <sup>(a)</sup>   | RoB Tier   |
|--|---|---|---|---|--|--|
|  |   |   |   |   | <p>area, outer diameter, section modulus, or buckling ratio were observed between the groups, apart from the following <u>in girls at the narrow neck</u>:</p> <p>Outer diameter, change over 1 yr (%), mean ± SE<br/>                     G1: 2.77 ± 0.50<br/>                     G2: 0.80 ± 0.48<br/>                     G3: 1.97 ± 0.51<br/>                     P = 0.02 for difference in change between groups. <sup>4)</sup></p> <p>Buckling ratio, change over 1 yr (%), mean ± SE<br/>                     G1: -1.98 ± 1.32<br/>                     G2: -6.53 ± 1.26<br/>                     G3: -4.16 ± 1.34<br/>                     P = 0.049 for difference in change between groups. <sup>4)</sup></p> |  |
| El-Hajj Fuleihan et al. (2006)<br>Lebanon<br>Latitude [assumed] 33.9° N<br>1 yr<br>Private funding | RCT (parallel)<br>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<br>Premenarcheal (%)<br>G1: 14.5<br>G2: 20.7<br>G3: 25.5<br>Age (y)<br>All<br>G1: 13.6 ± 2.1<br>G2: 13.0 ± 2.1 | Vitamin D <sub>3</sub><br>Doses<br>G1: placebo<br>G2: vitamin D <sub>3</sub> 1400 IU/wk [35 µg/wk = 5 µg/d]<br>G3: vitamin D <sub>3</sub> 14 000 IU/wk [350 µg/wk = 50 µg/d]<br>Analysed doses:<br>Stated that the vitamin D concentration in the 3 | BMD and BMC; DXA.<br>****<br><b>BMD at baseline: NR</b><br><b>BMC at baseline (g) All</b><br>Total hip:<br>G1: 24.3 ± 5.4 | Data extracted: ITT<br>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 returned for follow-up visits and BMD measurements were all  | <b>1 for females;<br/>                     2 for males</b> |



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

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

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

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

| Reference Study<br>Country<br>Duration<br>Funding | Design  | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D   | Results <sup>(a)</sup>  | RoB Tier |
|---|---|---|---|--|---|----------|
|   | <p>&lt;2.10 mmol/L; vitamin D supplement use &gt;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).</p> <p>N participants, randomised/ completed/analysed<br/>G1: 109/100/105<br/>G2: 100/94/97<br/>G3: 102/93/101</p> | <p>G2: 28.1 ± 5.0<br/>G3: 27.1 ± 4.1</p> <p>Ethnicity (%)<br/>Non-Hispanic White:<br/>G1: 93.3<br/>G2: 96.9<br/>G3: 97.0<br/>Non-Hispanic Black:<br/>G1: 1.0<br/>G2: 1.0<br/>G3: 0<br/>Asian:<br/>G1: 4.7<br/>G2: 2.1<br/>G3: 2.0<br/>Hispanic:<br/>G1: 1.0<br/>G2: 0<br/>G3: 1.0</p> <p>Smokers (%)<br/>G1: 1.9<br/>G2: 0<br/>G3: 4.0</p> <p>Alcohol use: NR</p> <p>Health status: NR</p> <p>Season: Aug 2013 to Dec 2017.</p> | <p>Background calcium intake: NR</p> <p><u>Compliance</u></p> <p>Pill count method – number of days of supplement administration vs total number of days (%)<br/>All: 99 on average, (range 81–100).</p> <p>Serum 25(OH)D at 36 mo (nmol/L)<br/>G1: 77.4 ± 17.9<br/>G2: 132.2 ± 28.0<br/>G3: 144.4 ± 40.4</p> | <p>G3: 329.7 ± 60.0<br/>Total, Tibia:<br/>G1: 301.2 ± 58.3<br/>G2: 314.1 ± 52.9<br/>G3: 306.5 ± 52.6<br/>Trabecular, Radius:<br/>G1: 163.1 ± 40.3<br/>G2: 160.4 ± 39.9<br/>G3: 155.9 ± 40.2<br/>Trabecular, Tibia:<br/>G1: 176.4 ± 37.7<br/>G2: 174.8 ± 35.2<br/>G3: 171.9 ± 38.7<br/>Cortical, Radius:<br/>G1: 887.6 ± 50.0<br/>G2: 899.2 ± 51.3<br/>G3: 904.0 ± 53.3<br/>Cortical, Tibia:<br/>G1: 853.9 ± 61.5<br/>G2: 868.6 ± 52.9<br/>G3: 871.5 ± 59.0</p> <p><b>Failure load at baseline</b><br/>Radius:<br/>G1: 2700.7 ± 1020.7<br/>G2: 2580.1 ± 990.4<br/>G3: 2556.5 ± 964.4<br/>Tibia:<br/>G1: 7831.3 ± 2420.1</p> | <p>G3: 317.3 ± 61.7<br/>Total, Tibia:<br/>G1: 299.1 ± 58.7<br/>G2: 309.1 ± 54.8<br/>G3: 301.5 ± 54.6<br/>Trabecular, Radius:<br/>G1: 164.8 ± 42.5<br/>G2: 161.5 ± 42.0<br/>G3: 155.1 ± 40.1<br/>Trabecular, Tibia:<br/>G1: 179.1 ± 40.3<br/>G2: 178.3 ± 39.1<br/>G3: 175.2 ± 40.8<br/>Cortical, Radius:<br/>G1: 879.6 ± 51.0<br/>G2: 886.3 ± 52.4<br/>G3: 884.9 ± 54.2<br/>Cortical, Tibia:<br/>G1: 848.1 ± 68.8<br/>G2: 856.6 ± 62.9<br/>G3: 853.7 ± 67.9</p> <p><b>vBMD, change over 36 mo (mg/cm<sup>3</sup>), mean, 95% CI</b><br/>Total, Radius<br/>G2 vs G1: -3.9, -6.5 to -1.3<br/>G3 vs G1: -7.5, -10.1 to -5.0<br/><i>P</i> &lt; 0.001 for group x time interaction.</p> |          |

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



| Reference Study<br>Country<br>Duration<br>Funding                            | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D  | Results <sup>(a)</sup>   | RoB Tier |
|--|--|--|---|---|--|----------|
|  |  |  |   |   | <p><math>P = 0.004</math> for group x time interaction.</p> <p><b>Failure load at 36 mo</b><br/>Radius:<br/>G1: <math>2694.8 \pm 1022.7</math><br/>G2: <math>2550.5 \pm 994.3</math><br/>G3: <math>2470.6 \pm 1001.5</math><br/><math>P = 0.12</math> for group x time interaction.</p> <p>Tibia:<br/>G1: <math>7785.2 \pm 2443.7</math><br/>G2: <math>7576.9 \pm 2125.0</math><br/>G3: <math>7413.0 \pm 2265.1</math><br/><math>P = 0.06</math> for group x time interaction.</p> |          |
| Grimnes et al. (2012)<br>Norway<br>Latitude 69° N<br>1 yr<br>Private funding | RCT (parallel)<br><br>Inclusion criteria:<br>Postmenopausal women aged 50–80 years old with a T-score in total hip or lumbar spine (L2–4) $\leq -2.0$ .<br><br>Exclusion criteria:<br>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<br><br>Age (y)<br>G1: $63.5 \pm 6.8$<br>G2: $62.9 \pm 7.6$<br><br>Serum 25(OH)D (nmol/L)<br>G1: $71.2 \pm 22.3$<br>G2: $70.7 \pm 23.0$<br>BMI (kg/m <sup>2</sup> )<br>G1: $24.6 \pm 3.2$<br>G2: $25.0 \pm 3.4$<br><br>Ethnicity [assumed]: Majority Caucasian | Vitamin D <sub>3</sub><br><br>Doses<br>G1: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + calcium 1000 mg/d<br>G2: vitamin D <sub>3</sub> 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<br><br>Background vitamin D intake (µg/d)<br>G1: $8.1 \pm 6.0$<br>G2: $9.1 \pm 6.2$ | BMD; DXA.<br><br>****<br><b>BMD at baseline (g/cm<sup>2</sup>)</b><br>Total hip:<br>G1: $0.791 \pm 0.082$<br>G2: $0.790 \pm 0.073$<br><br>Lumbar spine (L2-L4):<br>G1: $0.902 \pm 0.079$<br>G2: $0.901 \pm 0.072$<br><br>Femoral neck<br>G1: $0.757 \pm 0.079$<br>G2: $0.758 \pm 0.066$ | Data extracted: ITT<br><br><b>BMD, change over 12 mo (%)</b><br>Total hip:<br>G1: $0.56 \pm 1.70$<br>G2: $0.31 \pm 1.59$<br>$P = 0.20$ for difference in change between groups.<br><br>Lumbar spine (L2-L4):<br>G1: $0.32 \pm 3.23$<br>G2: $0.25 \pm 3.19$ .<br>$P = 0.86$ for difference in change between groups.  | <b>1</b> |

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

| Reference Study<br>Country<br>Duration<br>Funding                        | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D   | Results <sup>(a)</sup>   | RoB Tier |
|--|---|--|--|--|--|----------|
| Jorde et al. (2010)<br>Norway<br>Latitude 70° N<br>1 yr<br>Mixed funding | RCT (parallel)<br><br>Inclusion criteria:<br>Males and females 21–70 y, with BMI 28.0–47.0 kg/m <sup>2</sup> .<br><br>Exclusion criteria:<br>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)<br>G1: 61.0<br>G2: 59.8<br>G3: 60.0<br><br>Postmenopausal (%)<br>G1: 55%<br>G2: 48%<br>G3: 44%<br>Age (y)<br>G1: 50.8 ± 10.7<br>G2: 47.7 ± 11.6<br>G3: 47.3 ± 11.1<br><br>Serum 25(OH)D (nmol/L)<br>G1: 60.1 ± 22.3<br>G2: 58.3 ± 21.2<br>G3: 61.3 ± 20.7<br>BMI (kg/m <sup>2</sup> )<br>G1: 35.2 ± 3.9<br>G2: 33.7 ± 3.5<br>G3: 34.4 ± 3.9<br><br>Ethnicity [assumed]: Majority Caucasian<br>Smokers (%)<br>G1: 17.1<br>G2: 20.6<br>G3: 20.9<br><br>Alcohol use: NR | Vitamin D <sub>3</sub><br><br>Doses<br>G1: placebo + calcium 500 mg/d<br>G2: vitamin D <sub>3</sub> 20 000 IU/wk [2857 IU/d = 71.4 µg/d] + calcium 500 mg/d<br>G3: vitamin D <sub>3</sub> 40 000 IU [5714 IU/d = 142.9 µg/d] + calcium 500 mg/d<br><br>Background vitamin D intake: NR<br><br>Background calcium intake: NR<br><br><u>Compliance</u><br><br>Pill count method – vitamin D/placebo capsules (%)<br>G1: 96<br>G2: 96<br>G3: 95<br><br>Pill count method – calcium tablets (%)<br>G1: 83<br>G2: 84<br>G3: 82<br><br>Serum 25(OH)D at 1 yr (nmol/L)<br>G1: 57.9 ± 20.4 | BMD; DXA.<br><br>****<br>BMD at baseline (g/cm <sup>2</sup> )<br>Total hip:<br>G1: 1.092 ± 0.130<br>G2: 1.067 ± 0.128<br>G3: 1.107 ± 0.133<br><br>Lumbar spine (L2-L4):<br>G1: 1.251 ± 0.170<br>G2: 1.235 ± 0.161<br>G3: 1.270 ± 0.155<br><br>****<br>Assay of serum 25(OH)D: RIA (DiaSorin) | Data extracted: PP<br><br><b>BMD, change over 1 yr (g/cm<sup>2</sup>)</b><br>Total hip<br>G1: 0.009 ± 0.017<br>G2: 0.011 ± 0.014<br>G3: 0.008 ± 0.014<br><br>Lumbar spine (L2-L4):<br>G1: 0.007 ± 0.042<br>G2: 0.008 ± 0.039<br>G3: 0.008 ± 0.036<br>P=NS for all comparisons vs G1 (exact values NR). | <b>1</b> |

| Reference Study<br>Country<br>Duration<br>Funding                                     | Design   | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>  | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D   | Results <sup>(a)</sup>   | RoB Tier |
|---|--|--|--|--|--|----------|
|   | contraceptive rods, contraceptive dermal patches, injectable contraceptives, vaginal contraceptive rings, or intrauterine devices, with pearl index <1.0).<br><br>N participants randomised/ completed/analysed:<br>G1: 142/105/105<br>G2: 132/97/97<br>G3: 147/110/110  | Health status: Healthy overweight.<br>Season: Recruitment occurred in Nov 2005 to October 2006.  | G2: 99.7 ± 20.3<br>G3: 140.9 ± 34.7  |  |  |          |
| Rahme et al. (2017)<br>Lebanon<br>Latitude [assumed] 33.9° N<br>1 yr<br>Mixed funding | RCT (parallel)<br><br>Inclusion criteria:<br>Ambulatory elderly, aged ≥65 y; overweight (BMI >25 kg/m <sup>2</sup> ), serum 25(OH)D between 10 and 30 ng/mL [25 and 75 nmol/L] at screening.<br><br>Exclusion criteria:<br>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)<br>G1: 53.6<br>G2: 57.3<br><br>Age (y)<br>G1: 71.0 ± 4.7<br>G2: 71.2 ± 4.8<br><br>Serum 25(OH)D (ng/mL)<br>G1: 20.0 ± 7 ng/mL [50.0 ± 17.5 nmol/L]<br>G2: 20.9 ± 8.2 ng/mL [52.3 ± 20.5 nmol/L]<br><br>BMI (kg/m <sup>2</sup> )<br>G1: 29.7 ± 4.6<br>G2: 30.6 ± 4.4<br><br>Ethnicity [assumed]: Arab | Vitamin D <sub>3</sub><br><br>Doses (labeled)<br>G1: placebo + vitamin D <sub>3</sub> 500 IU/d [12.5 µg/d] + calcium 1000 mg/d<br>G2: vitamin D <sub>3</sub> 20 000 IU/wk [500 µg/wk] + vitamin D <sub>3</sub> 500 IU/d [12.5 µg/d] + calcium 1000 mg/d = vitamin D <sub>3</sub> 3357 IU/d [83.9 µg/d] + calcium 1000 mg/d<br><br>Analysed vitamin D <sub>3</sub> doses:<br>G1: 600 IU/d [15 µg/d]<br>G2: 22 000 IU/wk + 600 IU/d [15 µg/d] = 3742.86 IU/d [93.6 µg/d] | BMD; DXA.<br><br>****<br><b>BMD at baseline (g/cm<sup>2</sup>)</b><br>Total hip:<br>G1: 0.826 ± 0.119<br>G2: 0.824 ± 0.118<br><br>Lumbar spine:<br>G1: 0.886 ± 0.154<br>G2: 0.890 ± 0.150<br><br>Femoral neck:<br>G1: 0.676 ± 0.102<br>G2: 0.671 ± 0.101<br><br>****<br>Assay of serum 25 (OH)D: LC-MS (Mayo Clinic, | <u>Data extracted:</u> PP<br><br><b>BMD at 12 mo (g/cm<sup>2</sup>)</b><br>Total hip:<br>G1: 0.831 ± 0.121<br>G2: 0.828 ± 0.118<br><br>Lumbar spine:<br>G1: 0.901 ± 0.162<br>G2: 0.900 ± 0.145<br>Femoral neck:<br>G1: 0.682 ± 0.100<br>G2: 0.675 ± 0.099<br><br><b>BMD, change over 12 mo (%)</b><br>Total hip:<br>G1: 0.50 ± 2.26<br>G2: 0.47 ± 2.22 | <b>1</b> |

| Reference Study<br>Country<br>Duration<br>Funding                    | Design  | Subject characteristics at baseline <sup>(a)</sup>   | Intervention <sup>(a)</sup>   | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D | Results <sup>(a)</sup>  | 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:<br>G1: 128/112/112<br>G2: 129/110/110 | Smoking status: NR<br>Alcohol use: NR<br>Health status: Overweight<br>Season: Screening occurred between Jan 2011 and July 2013; the study ended in July 2014. | Background vitamin D intake: NR<br>Background calcium intake (mg/d)<br>G1: 453 ± 323<br>G2: 397 ± 257<br>Background calcium + vitamin D supplement use (%)<br>G1: 11 ± 10<br>G2: 11 ± 10<br><u>Compliance</u><br>Pill count method (%)<br>All: >90 for both calcium and vitamin D pills, for both groups.<br><br>Serum 25(OH)D at 12 mo (ng/mL)<br>G1: 25.9 ± 6.9 ng/mL [64.8 ± 17.3 nmol/L]<br>G2: 36.0 ± 9.7 ng/mL [90.0 ± 24.3 nmol/L] | Rochester, MN, USA)  | <i>P</i> = 0.909 for difference in % change between groups.<br><br>Lumbar spine:<br>G1: 1.34 ± 3.42<br>G2: 1.65 ± 3.21<br><i>P</i> = 0.512 for difference in % change between groups.<br><br>Femoral neck:<br>G1: 0.55 ± 3.78<br>G2: 0.66 ± 4.16<br><i>P</i> = 0.833 for difference in % change between groups. |          |
| Smith et al. (2018)<br><b>ViDOS</b><br>USA<br>Latitude 41° N<br>1 yr | RCT (parallel)<br><br>Inclusion criteria: Caucasian and African American postmenopausal women aged 57 to 90 years; vitamin D insufficiency  | Sex: Females<br><br>Age (y)<br>Caucasian: 67 ± 7.3<br>African American: 65 ± 7.0   | Vitamin D <sub>3</sub><br><br>Doses (labeled)<br>G1: placebo + calcium<br>G2: vitamin D <sub>3</sub> 400 IU/d [10 µg/d] + calcium<br>G3: vitamin D <sub>3</sub> 800 IU/d [20 µg/d] + calcium  | BMD; DXA<br><br>****<br><br>BMD at baseline: NR<br><br>****  | <u>Data extracted:</u> unclear<br><br>Caucasian and African American combined:<br><b>BMD, change over 1 yr (%), mean ± SE</b><br>Femoral neck:  | <b>1</b> |

| Reference Study<br>Country<br>Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup>  | Intervention <sup>(a)</sup>   | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D | Results <sup>(a)</sup>  | RoB Tier |
|---|--|---|---|--|---|----------|
| Mixed funding                                     | <p>defined as serum 25(OH)D ≤ 50 nmol/L.</p> <p>Exclusion criteria:<br/>Any significant disease or medications affecting calcium and bone metabolism:<br/>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<sup>2</sup>, 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</p> | <p>Serum 25(OH)D (nmol/L)<br/>Caucasian: 49.3 ± 13.3<br/>African American: 45.0 ± 14.8</p> <p>BMI: NR<br/>Ethnicity:<br/>Caucasian 84%<br/>African American 16%</p> <p>Smoking status: NR<br/>Alcohol use: NR<br/>Health status: NR</p> <p>Season: Enrolment in winter and spring over 2 years: Apr to May 2007 and Jan to May 2008.</p> <p>[Baseline characteristics published in the earlier publications could not be utilized here, as the results were combined regarding the ethnicities]</p> | <p>G4: vitamin D<sub>3</sub> 1600 IU/d [40 µg/d] + calcium<br/>G5: vitamin D<sub>3</sub> 2400 IU/d [60 µg/d] + calcium<br/>G6: vitamin D<sub>3</sub> 3200 IU/d [80 µg/d] + calcium<br/>G7: vitamin D<sub>3</sub> 4000 IU/d [100 µg/d] + calcium<br/>G8: vitamin D<sub>3</sub> 4800 IU/d [120 µg/d] + calcium</p> <p>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.</p> <p>Analysed vitamin D doses<br/>G2: 503 IU/d [12.6 µg/d]<br/>G3: 910 IU/d [22.8 µg/d]<br/>G4: 1532 IU/d [38.3 µg/d]<br/>G5: 2592 IU/d [64.8 µg/d]<br/>G6: 2947 IU/d [73.7 µg/d]<br/>G7: 4209 IU/d [105.2 µg/d]<br/>G8: 4937 IU/d [123.4 µg/d]</p> <p>Background vitamin D intake (IU/d)</p> | <p>Assay of serum 25(OH)D: LC-MS</p>   | <p>G1: -0.32 ± 0.80<br/>G2: 1.89 ± 0.85<br/>G3: 0.23 ± 0.79<br/>G4: 1.06 ± 0.77<br/>G5: -0.26 ± 0.77<br/>G6: 2.20 ± 0.82<br/>G7: 0.72 ± 0.83<br/>G8: 0.53 ± 0.82</p> <p><i>P</i> = 0.19 for difference in change between groups. <sup>3)</sup></p> <p>Lumbar spine (L1-L4):<br/>G1: 0.06 ± 0.63<br/>G2: -0.37 ± 0.67<br/>G3: 0.24 ± 0.62<br/>G4: 0.41 ± 0.60<br/>G5: 0.38 ± 0.60<br/>G6: 0.33 ± 0.67<br/>G7: 0.25 ± 0.63<br/>G8: 2.03 ± 0.64</p> <p><i>P</i> = 0.25 for difference in change between groups. <sup>3)</sup></p> <p><sup>3)</sup> adjusted for ethnicity.</p> |          |



| Reference Study<br>Country<br>Duration<br>Funding | Design   | Subject characteristics at baseline <sup>(a)</sup> | Intervention <sup>(a)</sup>  | Endpoint assessed; method of measurement<br>Endpoint at baseline <sup>(a)</sup> Assay of serum 25(OH)D | Results <sup>(a)</sup> | RoB Tier |
|---|--|--|--|--|------------------------|----------|
|   | phenytoin or phenobarbital, and high-dose thiazide therapy (>37.5 mg/d).<br><br>N participants, randomised/completed/analysed<br>Caucasian:<br>G1: 21/18/19<br>G2: 20/16/18<br>G3: 21/17/18<br>G4: 20/19/18<br>G5: 21/20/20<br>G6: 20/18/18<br>G7: 20/16/16<br>G8: 20/18/18<br>African American:<br>G1: 3/3/3<br>G2: 2/2/2<br>G3: 7/4/4<br>G4: 5/5/5<br>G5: 4/4/4<br>G6: 3/3/3<br>G7: 4/4/4<br>G8: 3/3/3 |  | Caucasian: 144 ± 69 IU/d [3.6 ± 1.7 µg/d]<br>African American: 129 ± 85 IU/d [3.2 ± 2.1 µg/d]<br>Background calcium intake (mg/d)<br>Caucasian: 685 ± 289<br>African American: 524 ± 156<br><br><u>Compliance</u><br><br>Pill count method - vitamin D <sub>3</sub> capsules (%):<br>All: 94 on average<br>Pill count method – calcium tablets (%):<br>All: 91 on average<br><br>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<sup>th</sup>–75<sup>th</sup> percentiles or the length between the percentiles.

## 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)<br>Hypercalcaemia, Hypercalciuria       | Gallagher et al. (2013); Gallagher et al. (2014a)<br>Hypercalcaemia, Hypercalciuria<br>Smith et al. (2017)<br>Falls<br>Smith et al. (2018)<br>BMD |
| Burt et al. (2019)<br>Bone fractures, Falls, BMD, bone strength | Billington et al. (2020)<br>Hypercalcaemia, Hypercalciuria  |
| Bischoff-Ferrari et al. (2020)<br>Bone fractures                | Bischoff-Ferrari et al. (2022)<br>Falls   |
| Jorde et al. (2008)<br>Hypercalcaemia, Hypercalciuria           | Sneve et al. (2008)<br>Hypercalcaemia, Hypercalciuria   |
| Macdonald et al. (2013)<br>Fractures                            | Wood et al. (2014)<br>Falls   |
| El-Hajj Fuleihan et al. (2006)<br>BMD, BMC                      | Al-Shaar et al. (2013)<br>BMD<br>Maalouf et al. (2008)<br>Hypercalcaemia  |

## 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.

- 1) Adebayo FA, et al. Vitamin D intake, serum 25-hydroxyvitamin D status and response to moderate vitamin D3 supplementation: a randomised controlled trial in East African and Finnish women. *British Journal of Nutrition*. 2018;119(4):431–441.
- 2) Aloia JF, et al. A randomized controlled trial of vitamin D3 supplementation in African American women. *Archives of Internal Medicine*. 2005;165(14):1618–1623.
- 3) Biancuzzo RM, et al. Fortification of orange juice with vitamin D(2) or vitamin D(3) is as effective as an oral supplement in maintaining vitamin D status in adults. *American journal of clinical nutrition*. 2010;91(6):1621–1626.
- 4) Cashman KD, et al. Dietary calcium does not interact with vitamin D3 in terms of determining the response and catabolism of serum 25-hydroxyvitamin D during winter in older adults. *American Journal of Clinical Nutrition*. 2014;99(6):1414–1423.
- 5) Cashman KD, et al. Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *American Journal of Clinical Nutrition*. 2012;95(6):1350–1356.
- 6) Cherniack EP, et al. The response of elderly veterans to daily vitamin D3 supplementation of 2,000 IU: a pilot efficacy study. *Journal of the American Geriatrics Society*. 2011;59(2):286–290.
- 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.
- 8) Czech-Kowalska J, et al. Impact of vitamin D supplementation during lactation on vitamin D status and body composition of mother-infant pairs: a MAVID randomized controlled trial. *PloS one*. 2014;9(9):e107708.
- 9) Gallagher JC, et al. Vitamin D supplementation in young White and African American women. *Journal of bone and mineral research*. 2014;29(1):173–181.
- 10) Gonnelli S, et al. Pharmacokinetic profile and effect on bone markers and muscle strength of two daily dosage regimens of calcifediol in osteopenic/osteoporotic postmenopausal women. *Aging Clinical and Experimental Research*. 2021;33(9):2539–2547.
- 11) Graeff-Armas LA, et al. Supplemental 25-Hydroxycholecalciferol Is More Effective than Cholecalciferol in Raising Serum 25-Hydroxyvitamin D Concentrations in Older Adults. *Journal of Nutrition*. 2020;150(1):73–81.
- 12) Himmelstein S, et al. Vitamin D supplementation in elderly nursing home residents increases 25(OH)D but not 1,25(OH)2D. *American Journal of Clinical Nutrition*. 1990;52(4):701–706.
- 13) Hunter D, et al. A randomized controlled trial of vitamin D supplementation on preventing postmenopausal bone loss and modifying bone metabolism using identical twin pairs. *Journal of Bone & Mineral Research*. 2000;15(11):2276–2283.
- 14) Iitkonen ST, et al. Effects of vitamin D2-fortified bread v. supplementation with vitamin D2 or D3 on serum 25-hydroxyvitamin D metabolites: an 8-week randomised-controlled trial in young adult Finnish women. *British Journal of Nutrition*. 2016;115(7):1232–1239.
- 15) Jorde R, et al. Effects of vitamin D supplementation on bone turnover markers and other bone-related substances in subjects with vitamin D deficiency. *Bone*. 2019;124:7–13.
- 16) Jorde R, et al. Prevention of urinary tract infections with vitamin D supplementation 20,000 IU per week for five years. Results from an RCT including 511 subjects. *Infectious Diseases*. 2016;48(11–12):823–828.
- 17) Karefylakis C, et al. Effect of Vitamin D supplementation on body composition and cardiorespiratory fitness in overweight men—a randomized controlled trial. *Endocrine*. 2018;61(3):388–397.
- 18) Lagari VS, et al. Differences in vitamin D3 dosing regimens in a geriatric community-dwelling population. *Endocrine Practice*. 2012;18(6):847–854.

- 19) Lehmann U, et al. Vitamin D3 supplementation: response and predictors of vitamin D3 metabolites - A randomized controlled trial. *Clinical nutrition*. 2016;35(2):351–358.
- 20) March KM, et al. Maternal vitamin D3 supplementation at 50 µg/d protects against low serum 25-hydroxyvitamin D in infants at 8 wk of age: a randomized controlled trial of 3 doses of vitamin D beginning in gestation and continued in lactation. *American Journal of Clinical Nutrition*. 2015;102(2):402–410.
- 21) Minisola S, et al. Correction of vitamin D status by calcidiol: pharmacokinetic profile, safety, and biochemical effects on bone and mineral metabolism of daily and weekly dosage regimens. *Osteoporosis International*. 2017;28(11):3239–3249.
- 22) Mitri J, et al. Effects of vitamin D and calcium supplementation on pancreatic beta cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *American journal of clinical nutrition*. 2011;94(2):486–494.
- 23) Nygaard B, et al. Effects of high doses of cholecalciferol in normal subjects: a randomized double-blinded, placebo-controlled trial. *PLoS ONE*. 2014;9(8):e102965.
- 24) Osmancevic A, et al. Vitamin D treatment in Somali women living in Sweden – Two randomized, placebo-controlled studies. *Clinical Endocrinology*. 2016;85(4):535–543.
- 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.
- 26) Rajakumar K, et al. Effect of vitamin D3 supplementation in black and in white children: A randomized, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism*. 2015;100(8):3183–3192.
- 27) Ryu OH, et al. A prospective randomized controlled trial of the effects of vitamin D supplementation on long-term glycemic control in type 2 diabetes mellitus of Korea. *Endocrine Journal*. 2014;61(2):167–176.
- 28) Toss G, et al. Is a daily supplementation with 40 microgram vitamin D3 sufficient? A randomised controlled trial. *European Journal of Nutrition*. 2012;51(8):939–945.
- 29) Vaes AMM, et al. Dose–response effects of supplementation with calcifediol on serum 25-hydroxyvitamin D status and its metabolites: a randomized controlled trial in older adults. *Clinical nutrition*. 2018;37(3):808–814.
- 30) Yao P, et al. A dose–response study of vitamin D3 supplementation in healthy Chinese: a 5-arm randomized, placebo-controlled trial. *European journal of nutrition*. 2016;55(1):383–392.
- 31) Zwart SR, et al. A 250 µg/week dose of vitamin D was as effective as a 50 µg/d dose in healthy adults, but a regimen of four weekly followed by monthly doses of 1250 µg raised the risk of hypercalciuria. *British Journal of Nutrition*. 2013;110(10):1866–1872.

For sQ4c – BMD/BMC.

- 1) Aloia JF, et al. A randomized controlled trial of vitamin D3 supplementation in African American women. *Archives of internal medicine*. 2005;165(14):1618–1623.
- 2) Andersen R, et al. Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-controlled intervention study. *British Journal of Nutrition*. 2008;100(1):197–207.
- 3) Balasuriya CND, et al. Maternal serum retinol, 25(OH)D and 1,25(OH)2D concentrations during pregnancy and peak bone mass and trabecular bone score in adult offspring at 26-year follow-up. *PLoS ONE [Electronic Resource]*. 2019;14(9):e0222712.
- 4) Bevilacqua G, et al. Determinants of circulating 25-hydroxyvitamin D concentration and its association with musculoskeletal health in midlife: Findings from the Hertfordshire Cohort Study. *Metabolism Open*. 2021;12:100143.
- 5) Breen ME, et al. 25-hydroxyvitamin D, insulin-like growth factor-I, and bone mineral accrual during growth. *Journal of Clinical Endocrinology & Metabolism*. 2011;96(1):E89–E98.
- 6) Brustad N, et al. High-dose vitamin D supplementation in pregnancy and 25(OH)D sufficiency in childhood reduce the risk of fractures and improve bone mineralization in childhood: follow-up of a randomized clinical trial. *EClinicalMedicine*. 2022;43:101254.
- 7) Cauley JA, et al. Serum 25 hydroxyvitamin D, bone mineral density and fracture risk across the menopause. *Journal of Clinical Endocrinology & Metabolism*. 2015;100(5):2046–2054.

- 8) Chan R, et al. Serum 25-hydroxyvitamin D, bone mineral density, and non-vertebral fracture risk in community-dwelling older men: results from Mr. Os, Hong Kong. *Archives of Osteoporosis*. 2011;6:21–30.
- 9) 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.
- 10) Dawson-Hughes B, et al. Rates of bone loss in postmenopausal women randomly assigned to one of two dosages of vitamin D. *American journal of clinical nutrition*. 1995;61(5):1140–1145.
- 11) del Puente A, et al. Dietary calcium intake and serum vitamin D are major determinants of bone mass variations in women. A longitudinal study. *Aging-Clinical & Experimental Research*. 2002;14(5):382–388.
- 12) Dennison E, et al. Determinants of bone loss in elderly men and women: a prospective population-based study. *Osteoporosis International*. 1999. 10:384–391.
- 13) Ensrud KE, et al. Serum 25-hydroxyvitamin D levels and rate of hip bone loss in older men. *Journal of Clinical Endocrinology & Metabolism*. 2009;94(8):2773–2780.
- 14) Garcia AH, et al. 25-hydroxyvitamin D concentrations during fetal life and bone health in children aged 6 years: a population-based prospective cohort study. *The Lancet Diabetes & Endocrinology*. 2017;5(5):367–376.
- 15) Hauksson HH, et al. Vitamin D status of Icelandic children and its influence on bone accrual. *Journal of Bone & Mineral Metabolism*. 2016;34(5):580–586.
- 16) Hunter D, et al. A randomized controlled trial of vitamin D supplementation on preventing postmenopausal bone loss and modifying bone metabolism using identical twin pairs. *Journal of bone and mineral research*. 2000;15(11):2276–2283.
- 17) Hyde NK, et al. Maternal vitamin D and offspring trabecular bone score. *Osteoporosis International*. 2017;28(12):3407–3414.
- 18) Larsen AU, et al. The effect of high-dose vitamin D3 supplementation on bone mineral density in subjects with prediabetes. *Osteoporosis international*. 2018;29(1):171–180.
- 19) Lawlor DA, et al. Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. *Lancet*. 2013;381(9884):2176–2183.
- 20) LeBoff MS, et al. Effects of Supplemental Vitamin D on Bone Health Outcomes in Women and Men in the VITamin D and Omega-3 Trial (VITAL). *Journal of bone and mineral research*. 2020;35(5):883–893.
- 21) Lehtonen-Veromaa MK, et al. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. *American Journal of Clinical Nutrition*. 2002;76(6):1446–1453.
- 22) Nakamura K, et al. Nutritional and biochemical parameters associated with 6-year change in bone mineral density in community-dwelling Japanese women aged 69 years and older: The Muramatsu Study. *Nutrition*. 2012;28(4):357–361.
- 23) Nieves JW, et al. Skeletal effects of vitamin D supplementation in postmenopausal black women. *Calcified tissue international*. 2012;91(5):316–324.
- 24) Patel R, et al. The effect of season and vitamin D supplementation on bone mineral density in healthy women: a double-masked crossover study. *Osteoporosis international*. 2001;12(4):319–325.
- 25) Sayers A, et al. 25-Hydroxyvitamin-D3 levels are positively related to subsequent cortical bone development in childhood: findings from a large prospective cohort study. *Osteoporosis International*. 2012;23(8):2117–2128.
- 26) Steingrimsdottir L, et al. Hip fractures and bone mineral density in the elderly--importance of serum 25-hydroxyvitamin D. *PLoS ONE [Electronic Resource]*. 2014;9(3):e91122.
- 27) Swanson CM, et al. Associations of 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D With Bone Mineral Density, Bone Mineral Density Change, and Incident Nonvertebral Fracture. *Journal of bone and mineral research*. 2015;30(8):1403–1413.
- 28) Thompson M, et al. Population Vitamin D Stores Are Increasing in Tasmania, and This Is Associated With Less BMD Loss Over 10 Years. *Journal of Clinical Endocrinology & Metabolism*. 2021;106(8):e2995–e3004.
- 29) Yang Y, et al. The Association of Vitamin D in Youth and Early Adulthood with Bone Mineral Density and Microarchitecture in Early Adulthood. *Calcified Tissue International*. 2019;104(6):605–612.

- 30) Zhu K, et al. Effects of calcium and vitamin D supplementation on hip bone mineral density and calcium-related analytes in elderly ambulatory Australian women: a five-year randomized controlled trial. *Journal of clinical endocrinology and metabolism*. 2008;93(3):743–749.



## 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  $\mu\text{g}/\text{d}$  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<sub>3</sub>) and the achieved serum 25(OH)D concentrations per  $\mu\text{g}/\text{d}$  of vitamin D administered. A one-stage multivariate dose–response meta-analysis (Crippa et al., 2019) was set up.

First a linear shape was assumed including as fixed effect the dose of vitamin D ( $\mu\text{g}/\text{d}$ ), the form of administered vitamin D (calcidiol vs. vitamin D<sub>3</sub>) 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<sub>3</sub> 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:

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

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

$$I_{cal} \text{ indicator variable} = \begin{cases} 1 & \text{if arm with calcidiol} \\ 0 & \text{if arm with vitD}_3 \end{cases}$$

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

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

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 <sub>3</sub>   | 8.04     | 21.74 | –37.16         | 53.24          |
| Dose vitamin D <sub>3</sub>  | –0.07    | 0.23  | –0.55          | 0.42           |
| Intercept calcidiol (expressed as differential intercept from vitamin D <sub>3</sub> ) | 6.30     | 11.23 | –17.05         | 29.64          |
| Dose calcidiol (expressed as differential parameter from vitamin D <sub>3</sub> )      | –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<sub>3</sub> and calcidiol), the type of vitamin D administered, or baseline serum 25(OH)D concentrations on achieved serum 25(OH)D concentrations per  $\mu\text{g}/\text{day}$  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.

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

| Parameter  | Estimate | Se    | CI lower bound | CI Upper bound |
|--|----------|-------|----------------|----------------|
| Intercept vitamin D <sub>3</sub>   | 5.29     | 5.34  | –5.79          | 16.36          |
| Dose vitamin D <sub>3</sub>  | –0.07    | 0.23  | –0.55          | 0.42           |
| Intercept calcidiol (expressed as differential intercept from vitamin D <sub>3</sub> ) | 5.99     | 10.97 | –16.77         | 28.75          |
| Dose calcidiol (expressed as differential parameter from vitamin D <sub>3</sub> )      | –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 <sub>3</sub>   | 5.64     | 5.01 | –4.73          | 16.01          |
| Intercept calcidiol (expressed as differential intercept from vitamin D <sub>3</sub> ) | 4.09     | 4.75 | –5.73          | 13.92          |
| Dose vitamin D <sub>3</sub>  | –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 <sub>3</sub>  | –3.84    | 75.26 | –160.83        | 153.16         |
| Intercept calcidiol (expressed as differential intercept from vitamin D <sub>3</sub> )      | 15.78    | 78.03 | –146.99        | 178.56         |
| Dose vitamin D <sub>3</sub>   | 0.54     | 4.99  | –9.86          | 10.94          |
| Dose calcidiol (expressed as differential parameter from vitamin D <sub>3</sub> )           | –0.84    | 5.84  | –13.01         | 11.34          |
| Dose vitamin D <sub>3</sub> quadratic   | –0.007   | 0.06  | –0.14          | 0.12           |
| Dose calcidiol quadratic (expressed as differential parameter from vitamin D <sub>3</sub> ) | 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

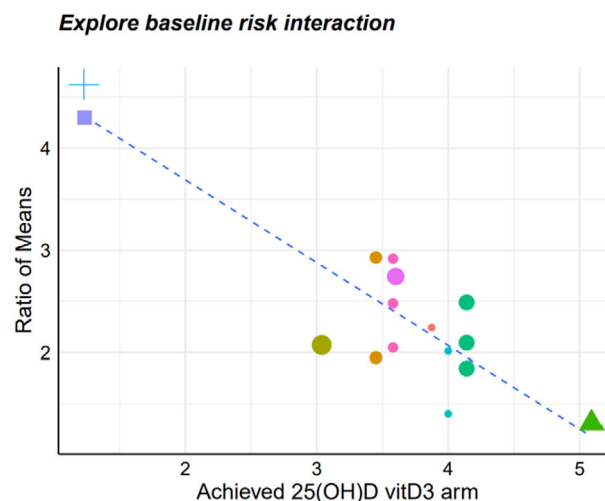
Funnel plots are traditionally used to explore whether publication bias could affect the body of evidence (Sterne et al., 2011) displaying the effect estimate standard error (SE) towards the study's effect (RoM between calcidiol and vitamin D<sub>3</sub> arms in this case). An asymmetric funnel indicates 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.

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<sub>3</sub> 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<sub>3</sub> is administered). Each point represents a comparison between calcidiol and vitamin D<sub>3</sub>. Arms from the same study share the same colour. The shape identifies the dose of vitamin D<sub>3</sub> administered; the size of the dots is indicative of the sample size of the comparator arm.

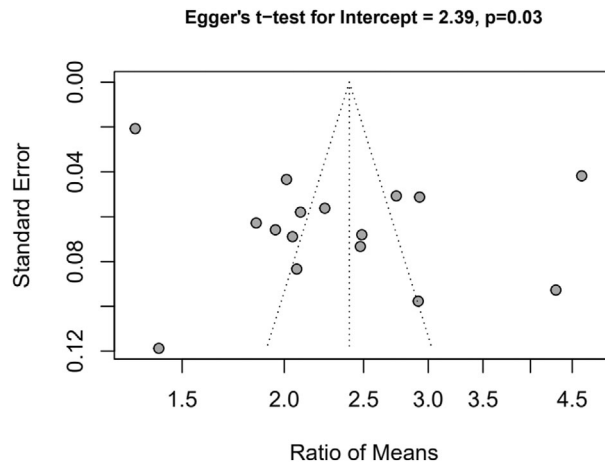
The plot shows a strong dependency of the RoM on the comparator level (the so-called baseline risk interaction) mainly driven by arms with high levels of vitamin D<sub>3</sub> as comparator (top left) and the arm with a dose of 25 µg/d (bottom right). They correspond to the dots in the extreme right 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<sub>3</sub>) 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<sub>3</sub>)

**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<sub>3</sub>).



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

## 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.<br>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).<br>The requirement shall not apply to cheese.<br>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) <sup>1</sup> 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   | 10 µg/100 g     |
|                 | Butter  | 10 µg/ 100 g    |
|                 | Milk (to consumers)   | 0,4 µg/100 g    |
| The Netherlands | Fats, liquid products with the same purpose, baking and frying products                             | Max. 0.075 µg/g |

| 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   |



| 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  |

| 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 |

## List of Annexes

These Annexes can be found in the online version of this output, under the section 'Supporting information', at: <https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2023.8145#support-information-section>

**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