Individual participant data (IPD)-level meta-analysis of randomised controlled trials with vitamin D-fortified foods to estimate Dietary Reference Values for vitamin D

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Abbreviations:

25(OH)D, 25-hydroxyvitamin D

AI, Adequate Intake

DRI, Dietary Reference Intake

DRV, Dietary Reference Values

EAR, Estimated Average Requirement

EFSA, the European Food Safety Authority

FFQ, Food Frequency Questionnaire

IOM, Institute of Medicine

IPD, Individual Participant Data

NNR, the Nordic Council of Ministers' Nordic Nutrition Recommendations

RCT, Randomized Controlled Trial

RDA, Recommended Dietary Allowance

RI, Recommended Intake

RNI, Reference Nutrient Intake

SACN, the Scientific Advisory Committee on Nutrition

UVB, Ultraviolet B

WHO-FAO, World Health Organisation–Food and Agriculture Organization

SR-MA, Systematic Reviews and Meta-Analyses

Abstract

Context and purpose: Individual participant data-level meta-regression (IPD) analysis is superior to meta-regression based on aggregate data in determining Dietary Reference Values (DRV) for vitamin D. Using data from randomized controlled trials (RCTs) with vitamin D₃-fortified foods, we undertook an IPD analysis of the response of serum 25-hydroxyvitamin (25(OH)D) to total vitamin D intake during winter among children and adults and derived DRV for vitamin D.

Methods: An IPD analysis using data from 1429 participants (ages 2-89 years) in 11 RCTs with vitamin D-fortified foods identified via a systematic review and predefined eligibility criteria. Outcome measures were vitamin D DRV estimates across a range of serum 25(OH)D thresholds using unadjusted and adjusted models.

Results: Our IPD-derived estimates of vitamin D intakes required to maintain 97.5% of winter 25(OH)D concentrations ≥ 25 and ≥ 30 nmol/L are 6 and 12 $\mu\text{g/day}$, respectively (unadjusted model). The intake estimates to maintain 90%, 95% and 97.5% of concentrations ≥ 50 nmol/L are 33.4, 57.5 and 92.3 $\mu\text{g/day}$, respectively (unadjusted) and 17.0, 28.1 and 43.6 $\mu\text{g/day}$, respectively (adjusted for mean values for baseline serum 25(OH)D, age and BMI).

Conclusions: IPD-derived vitamin D intakes required to maintain 90%, 95% and 97.5% of winter 25(OH)D concentrations ≥ 50 nmol/L are much higher than those derived from standard meta-regression based on aggregate data, due to the inability of the latter to capture between person-variability. Our IPD provides further evidence that using food-based approaches to achieve an intake of 12 $\mu\text{g/d}$ could prevent vitamin D deficiency (i.e., serum 25(OH)D < 30 nmol/L).

Keywords: Vitamin D recommendations; Dietary Reference Values; Recommended Dietary Allowance; Individual Participant Data-level meta-regression analyses; Vitamin D-fortified foods

Introduction

There is widespread acknowledgement of the presence of vitamin D deficiency in the general population [1-3]. A serum 25-hydroxyvitamin D (25(OH)D) concentration of 30 nmol/L represents a cut-off below which the risk of clinical vitamin D deficiency increases, manifesting as nutritional rickets in children and osteomalacia in adults [4]. The prevalence of serum 25(OH)D <30 nmol/L has been recently reported as 13%, 8.8% and 5.0% for representative population samples in Europe, Canada and the US, respectively [2,5,6]. These population-wide prevalence estimates, when coupled with current population size data, crudely suggest 20 and 97 million individuals in the US plus Canada and Europe, respectively, are at increased risk of clinical vitamin D deficiency. Thus, the first key priority from a public health perspective is to ensure that this risk is minimized [1,2]. It should be noted also that beyond deficiency, several [4,7,8], but not all [9], expert bodies briefed with development of dietary recommendations for vitamin D, using musculoskeletal health as the primary basis and some extra-skeletal health outcomes to a much lesser extent, proposed 50 nmol/L as the concentration of serum 25(OH)D that would meet the physiological vitamin D requirement of nearly all 'normal healthy persons'. While it has also been suggested that in terms of extra-skeletal health, serum 25(OH)D concentration should exceed 75 nmol/L [10], a number of recent umbrella reviews in the area [11-13] as well as the findings of some expert bodies [7,8], do not support the assertion that circulating 25(OH)D concentrations above 50 nmol/L are needed by all individuals for the prevention of non-skeletal diseases.

In the absence of sufficient ultraviolet B (UVB) radiation availability and/or exposure to enable vitamin D synthesis in the skin, dietary supply of vitamin D is critical to meeting population requirements and prevention of deficiency [14]. The median of average vitamin D intakes from various national nutrition surveys in Europe was ~3 µg/d [15,16] and ~7 µg/d in Canada and the US [17,18], highlighting how the majority of individuals in these regions, with the exception of some Nordic countries, are considered to have inadequate intakes [15,17,18]. This widespread dietary inadequacy is because there are very few rich natural food sources of vitamin D, the major ones being oily fish, egg yolks, and vitamin D-fortified products in some countries [14,15,18,19]. In terms of potential strategies for addressing inadequate micronutrient intake, the World Health Organisation–Food and Agriculture Organization (WHO–FAO) have suggested that food fortification has the widest and most

sustained impact, and thus represents a very cost-effective public health intervention [20].

Importantly, two systematic reviews and meta-analyses (SR-MA) have provided a key evidence-base for the efficacy of food fortification in adults in the form of data from up to 16 separate randomized controlled trials (RCTs) from around the world [21,22]. While these provide evidence at the highest level that food fortification increases serum 25(OH)D in a RCT setting, 12 of the 16 RCTs used dairy products as a food vehicle and 9 of these were milk/milk powder-based [21]. The valuable contribution vitamin D-fortified milk makes to vitamin D intakes, particularly in children, and the continued need for vitamin D fortification of milk and other dairy products is widely acknowledged [23,24]. However, it has been suggested that vitamin D fortification of a wider range of foods, which accommodate diversity, is likely to have the potential to increase vitamin D intakes across the population distribution and minimize the prevalence of low serum 25(OH)D [21,23]. Since the publication of the two SR-MA (7 and 12 y ago [21,22]), and particularly considering the diversification of food vitamin D fortification beyond milk, several new RCTs of vitamin D-fortified foods have been published [reviewed in 14,25]. A recent SR-MA of vitamin D RCTs in children (ages 2-18 y) reported a significantly greater response of serum 25(OH)D per 2.5 µg vitamin D₃ increment/d in trials using fortified foods compared to those with daily vitamin D supplements [26]. While the reason for this finding is not known, it is of note, as internationally the Dietary Reference Values (DRV) or equivalents for vitamin D have for the most part been established using dose-response data from RCTs with vitamin D₃ supplements [4,7-9]. Increasingly, the use of individual participant data (IPD)-level meta-regression analysis is recognized as best practice [27], as it avoids some of the limitations intrinsic to standard meta-regression based on aggregate data [28,29]. None of the three SR-MA [21,22,26] had access to individual data from the included RCTs so they were unable to undertake IPD-level meta-regression. Similarly, most recent DRV for vitamin D were based on standard meta-regression of aggregate data [4,7,8], mainly from supplementation trials.

Thus, the aims of the present work were firstly, through the process of a systematic review, to identify RCTs with vitamin D₃-fortified foods and subsequently use their individual data to undertake a priority IPD meta-regression analyses of the response of winter serum 25(OH)D to total vitamin D₃ intake in both children and adults. Secondly, to compare our IPD-derived

vitamin DRV estimates based on vitamin D₃-fortified food RCTs with international DRV which were largely based on vitamin D₃-supplement RCTs; as well as comparing these estimates with those from our previous IPD (also based on vitamin D₃-supplement RCTs).

Materials and methods, including scientific approach

The methodology in the present work follows the general methodology for systematic reviews as well as the more specialized IPD meta-regression analysis in the area of the vitamin D intake-serum 25(OH)D response applied previously [19,28,30-32], with brief details as follows:

Adherence to IPD guidelines and registration

The present IPD meta-regression analysis of data from food-based vitamin D RCTs follows the guidance provided as part of the PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*)-IPD statement [33]. The overall process can be considered a set of sequential steps starting with a systematic review to identify the appropriate vitamin D RCTs and culminating in statistical analyses that estimates the dose-response relationship, and hence the DRV for vitamin D, utilizing the pooled data of individual participants from all included RCTs [28,29,34].

The IPD meta-regression analysis was registered with the PROSPERO International Prospective Register of Systematic Reviews (registration number: CRD42018097260; https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=97260).

Ethics approval

Approval by a research ethics committee to conduct this meta-analysis was not required because the aim of this secondary analysis was consistent with the ethical approval received for the individual studies. The current analysis was conducted on anonymized data.

Systematic review to identify eligible papers

Eligibility criteria

The following outlines eligibility criteria for the food-based vitamin D RCTs for inclusion in the IPD-level meta-regression analysis to estimate the DRV for vitamin D. As with our previous IPD [28], the current IPD followed an approach that prioritized the identification of the vitamin D intake values to maintain serum 25(OH)D concentrations above chosen cut-offs when UVB-induced skin synthesis of vitamin D is absent or markedly diminished. This is closely aligned with the predefined criteria used by the Institute of Medicine (IOM) in their 2011 *Vitamin D and Calcium Dietary Reference Intake* (DRI) exercise [4] to select RCTs considered most appropriate to address the specific question of setting dietary requirements for vitamin D to meet pre-specified 25(OH)D thresholds and is similar to that taken by the Nordic Council of Ministers' *Nordic Nutrition Recommendations* (NNR) [8], the Scientific Advisory Committee on Nutrition (SACN) in the UK [9], and the European Food Safety Authority (EFSA) [7].

Within the Population Intervention Comparison Outcome (PICO) framework [35], the *populations* of interest in this study were specified as male and female children and adults, but excluding studies in infants (0-12 months) and young toddlers (12-23.9 months), pregnant or lactating women, and dark-skinned individuals (defined as those with a Fitzpatrick skin type of V or VI [34]). These excluded population subgroups have physiological considerations in relation to vitamin D [28] which require dedicated IPD meta-regression analyses [29,34]. Studies on animals and patient groups with diseases that are assumed to affect vitamin D metabolism and/or response to vitamin D₃ supplementation (see **Supplemental Table 1** in 'Online Resource', as per [28,35]) were excluded. Relevant food-based vitamin D RCTs were defined as those fulfilling the following characteristics:

- (1) *Intervention*: vitamin D₃ consumed orally as a fortified/enhanced/enriched food(s) and taken daily or weekly. RCTs which supplied vitamin D₃-fortified/enhanced/enriched food(s) less frequently than weekly (e.g., monthly, quarterly, annually) were excluded. The foods were to be consumed as part of a diet and not oral supplements, supplement sachets for addition to foods, or be fortified/enhanced/enriched food(s) supplying ≤ 100

IU/d (2.5 µg/d; 1 µg = 40 IU). Inclusion of RCT arms was limited to those with a maximum added vitamin D₃ dose of 4000 IU (100 µg)/d (or daily equivalent, in case of doses provided less frequently than daily). This selection of upper maximum dose takes account of the Tolerable Upper Intake Level (UL) for vitamin D of 4000 IU/d for those upwards of 9 and 11 y, set by EFSA [36] and IOM [4]. The selection of upper maximum dose of 4000 IU/d also allows for a trend amongst adults for increasing use of higher dose vitamin D supplements [37].

RCTs needed to use vitamin D₃, not vitamin D₂, on the basis that (i) the IOM DRI committee and EFSA used studies with vitamin D₃ in their regression analyses, to set DRV [4,7], and (ii) there is evidence that the relative potency of vitamin D₂ to increase serum total 25(OH)D is lower than vitamin D₃ [38,39]. While the IOM only selected studies that provided vitamin D alone and not with co-administration of calcium [4], both the NNR [8] and EFSA [9] allowed studies which co-administered calcium to be included. We have provided RCT data to suggest that calcium intake does not influence the response of serum 25(OH)D to vitamin D₃ supplementation [40] and DRV for vitamin D are established under the assumption that calcium intake is adequate [4,7-9]. Thus, we allowed food-based RCTs that provided vitamin D alone or in combination with calcium to be included. As foods were the delivery vehicle as opposed to supplements, we also allowed other micronutrients (e.g., vitamin K, B-vitamins, iron) to be included in addition to vitamin D and calcium.

- (2) RCTs using a food derived from a 'vitamin D-biofortification' approach, in which 25(OH)D₃ alone or in combination with vitamin D₃ was included in the feedstuffs for poultry, livestock or farmed fish, which was then incorporated into their tissues and thus in food for human consumption [25,41], were allowed. Otherwise, RCTs with vitamin D metabolites (25(OH)D and 1,25(OH)₂D) and analogues (e.g., alfacalcidol) as the human food fortificant were excluded.

- (3) *Outcome and comparator/comparison*: reported serum or plasma 25(OH)D concentration following supplementation in at least one vitamin D intervention group and one control/placebo group needed to be available. A conversion factor of 2.496 nmol/L = 1 ng/mL was used to standardise all serum or plasma concentrations to nmol/L. Studies with no data on measured serum or plasma 25(OH)D were excluded.
- (4) Only studies conducted at latitudes $\geq 40^{\circ}\text{N}$ during, or at least incorporating, winter, to ensure minimal impact of UVB on the vitamin D intake–25(OH)D dose-response, and thus the calculated vitamin D₃ intake requirements to achieve serum 25(OH)D thresholds, were included. EFSA in their recent vitamin D DRV analyses defined a period of assumed minimal endogenous vitamin D synthesis at latitudes $\geq 40^{\circ}\text{N}$ (covering much of Europe) as October through April [7]. The IOM and NNR selected RCTs performed in northern latitudes $>49.5/50^{\circ}\text{N}$ in Europe during winter-time as the dataset upon which to explore the vitamin D dose response relationship and establish their DRI [4,8]. Thus, we only included data from an RCT if it took place at a latitude greater than at least 40°N and entirely within the window of October and April, or had an intermediate sampling point within this winter period and of at least 6 weeks of vitamin D supplementation.
- (5) The study duration needed to be at least 6 weeks as serum 25(OH)D concentrations in adult and elderly subjects only reach equilibrium after 6–8 weeks of vitamin D supplementation [42]. Studies of a duration less than 6 weeks were excluded.
- (6) Assessment of vitamin D intakes, on which to base the dose-response calculations, was based on food frequency questionnaire (FFQ), dietary history, 24-hour recall for ≥ 3 d, or a food record or diary for ≥ 3 d, as per [35]. We used ‘total vitamin D intake’, which is the total vitamin D intake from the diet (including personal vitamin D supplements, where permitted within a RCT) as well as that from any supplemental vitamin D dose provided in the RCT [43,44]. The use of total vitamin D intake to derive DRV has been

prioritized by expert agencies and bodies [4,7-9]. Thus, RCTs that had not assessed habitual vitamin D intake in study participants were excluded.

Identification of studies: Information sources and search strategy

During May-July 2018, electronic searches were performed in the following three online databases (PubMed, Ovid Medline and Embase) as well as three trial registries (ClinicalTrials.gov, Cochrane Central Register of Controlled Trials (CENTRAL), and the International Standard Randomized Controlled Trials Number (ISRCTN) registry) from inception to July 31st 2018 (date of the final screen) using structured electronic search strategies which accounted for the inclusion/exclusion criteria outlined above. The search strategies were based closely on that used by us previously for identification of vitamin D RCTs that could inform establishment of dietary requirements [30,31], and an exemplar search strategy specifically adapted for PubMed is shown in **Supplemental Table 2** in ‘Online Resource’. The methods used in the present systematic review follow the PRISMA statement [45].

Study selection and inclusion

Study selection was independently conducted by two pre-specified investigators (KDC and MEK), first by a screen of the titles and abstracts, followed by a review of the full text of potentially relevant studies. The same two investigators separately determined which RCTs met the eligibility criteria and were included. In addition, the searches were supplemented by searches of review/systematic review articles and reference lists of trial publications as well as from the key international vitamin D DRV reports over the last 9 y [4,7-9]. Duplicates were removed in EndNote (X7.0.1, Clarivate Analytics, Philadelphia, Pennsylvania, United States). Studies that fulfilled the inclusion criteria and were not collected on the basis of the database search were added. Information on the combined number of records identified, abstracts and full-text articles screened, and articles excluded and included in the review are shown in **Figure 1**.

For each eligible RCT, collaboration was requested and negotiated with the principal investigator [46]. The details of data collection processes, including the requested data items, the checking of the IPD integrity as well as the data protection measures [47] are provided in the **Supplemental information on Methods** in the ‘Online Resource’.

Specification of outcomes and effect measures

Serum 25(OH)D concentration was the sole outcome considered in the IPD meta-regression analysis. Likewise, total vitamin D intake was the only predictor considered.

Quality assessment and risk of bias assessment for individual studies

The Jadad scale was used to assess the quality of the included RCTs [48], and an assessment of the risk of bias in these RCTs was performed using the Cochrane Collaboration’s tool for assessing risk of bias [49]. Further details on both assessments, including the pre-specified assessors, are provided in the Supplemental information on Methods in the ‘Online Resource’.

Statistics

Choice of model

A one-stage IPD meta-analysis was carried out [50,51]. Initially, both linear and non-linear regression models for describing the relationship between serum 25(OH)D and total vitamin D₃ intake were fitted [4,28,31] and, based on model diagnostics (residual and QQ plots), results from the most appropriate model were reported. When assuming a linear trend, the one-stage IPD meta-analysis corresponded to fitting a linear regression model with vitamin D intake as the fixed effect and study-specific random intercept and slope effects. When assuming a non-linear trend, the one-stage IPD meta-analysis was carried out by fitting a linear regression model with logarithm-transformed vitamin D intakes and serum 25(OH)D concentrations, corresponding to a non-linear power model on the original scales; this model provided an operational and reasonable approximation to the exponential models previously

used (as no levelling off at high intakes was observed). Both models without covariate adjustment and models including adjustment for baseline serum 25(OH)D concentrations, which were also logarithm-transformed in cases where the outcome was logarithm-transformed, age, and BMI were fitted. These were pre-specified covariates commonly used in this type of modelling exercise [7,28]. In sensitivity analyses we also carried out the adjusted analyses replacing BMI by body weight and *z*-scores for body weight or BMI (one at a time). Child age- and sex-specific weight and BMI standard deviation scores (*z*-scores) were generated using LMS growth software and the UK-WHO 0 to 4-year and UK 4 to 20-year growth reference data [52]. Likewise, additional adjustments for methods of vitamin D intake estimation or serum 25(OH)D measurement were also performed. All models included study-specific random intercepts, implying that linear mixed models were fitted (using restricted maximum likelihood estimation). Intake and covariate adjustment (if included) constituted the fixed effects in the models. The model which adjusted for baseline 25(OH)D, age and BMI will be referred to as the ‘adjusted model’ throughout the remainder of the paper, and where a model used alternate or additional adjustments these will be highlighted.

To inform safety considerations around each of the various vitamin D intake requirement estimates generated from the present IPD analysis, these were compared to the ULs for vitamin D from children (50-75 µg/d, depending on age group and agency) and adults (100 µg/d) [4,36]. In addition, the present unadjusted and adjusted models were used to predict the upper 97.5th percentile of serum 25(OH)D concentration achieved. While the serum 25(OH)D concentration representing the vitamin D toxicity threshold in humans is not readily defined [4], EFSA recently concluded that a serum 25(OH)D concentration of 200 nmol/L or below is unlikely to pose a risk of adverse health outcomes in healthy infants [53], a sensitive group within the population. The IOM, in setting their ULs for vitamin D, also considered if the intakes were likely to lead to serum 25(OH)D concentration in excess of approximately 125 to 150 nmol/L, which they considered might be of concern based on some observed U-shaped or reverse-J-shaped relationships between serum 25(OH)D and mortality as well as

other health outcomes [4]. However, EFSA considered that studies reporting on an association between 25(OH)D concentration and all-cause mortality or cancer were inconsistent and they did not raise this concern [7,36]. Thus, we benchmarked the upper 97.5th percentile of serum 25(OH)D concentrations against serum 25(OH)D concentrations of 150 and 200 nmol/L.

Derivation of vitamin D DRV estimates

Lower boundaries of the prediction intervals of the fitted (mean) regression line, corresponding to vitamin D intakes needed to maintain 50, 90, 95, and 97.5% of the participants above serum 25(OH)D thresholds of 25, 30, and 50 nmol/L (where appropriate and feasible) were estimated by means of inverse regression. While SACN's *Reference Nutrient Intake* (RNI) [9], NNR's *Recommended Intake* (RI) [8] and IOM's *Recommended Dietary Allowance* (RDA) [4] are all intended to meet the requirements of 97.5% of the individuals in the population, EFSA's *Adequate Intake* (AI) is intended as an intake at which most of the population will achieve the target serum 25(OH)D concentration [7]. Thus, the AI may not cover 97.5%, but 95% or some other majority percentage. Standard errors and 95% confidence intervals on these estimated lower boundaries were obtained using a parametric bootstrap procedure with 1000 replications, as described previously [28]. In order to achieve comparable fitted regression lines for unadjusted and adjusted model fits, we fixed covariates at their overall mean value (except for intake) in adjusted models.

Leave-one-out sensitivity analysis

To assess if there were any overly influential RCTs, the derivation of vitamin D DRV estimates (as described above) based on an unadjusted model based on data from all RCTs was repeated leaving out one RCT at a time. Absolute and relative changes in estimates as compared to the full analysis were reported.

Subgroup analyses

A number of specific subgroups had been considered previously [4,7,28]. Therefore we also carried out these subgroup analyses. Specifically, we fitted separate models for subgroups of children (<18 years) and adults (≥ 18 years) and for subgroups of participants whose baseline serum 25(OH)D was < or ≥ 50 nmol/L. We also fitted separate models for the subgroup of RCTs of children and adults which were $\geq 50^\circ\text{N}$; only models including adjustment for baseline serum 25(OH)D level, age, and BMI were fitted. Furthermore, we fitted separate models for the subgroup of RCTs of adults which provided vitamin D at a dose ≤ 30 $\mu\text{g/day}$, above which the rise in serum 25(OH)D to additional vitamin D is less steep [31]. We also fitted separate models for a subgroup consisting of adult participants with BMI <25 kg/m². Lastly, we did subgroup analysis for RCTs where compliance data were available and for participants showing compliance above 80%, and separately above 95%. The subgroup analyses were pre-specified rendering testing for interaction unnecessary.

Estimation of rate constants for included RCTs

It has been suggested that the bioavailability of vitamin D from food could potentially be influenced by the complexity of the food matrix [54]. However, data from the limited number of RCTs which compared vitamin D₃-fortified foods (regular and low-fat cheese, orange juice, various breads) with vitamin D₃ supplements demonstrate equal bioavailability [55-58]. Use of the ‘rate constant’ (i.e. the change in 25(OH)D in nmol/L per μg vitamin D administered [59]) would allow a comparison of vitamin D status improvement per dose of vitamin D from fortified foods and supplements across RCTs among individuals of different ages within the present IPD and equivalent winter-based RCTs with vitamin D supplements included in the IOM’s DRI exercise [4], respectively. Rate constants were calculated as described by Whiting *et al.* [59], namely, for both the control (placebo) group and the treatment group(s), the net change from baseline to endpoint 25(OH)D determined by subtraction. The rise (or fall) in 25(OH)D of the control group is subtracted (or added) to the net change of 25(OH)D in the treatment group(s). The resulting nmol/L is divided by the dose (in μg) of vitamin D₃ administered [59].

Statistical software

All analyses were conducted using R version 3.6.2 (R Core Team, Vienna, Austria) and the R extension packages “boot” for bootstrapping; “medrc”, “lme4” [60] and “nlme” [61] for fitting linear mixed models. The R code for fitting linear and nonlinear models is presented elsewhere [34].

RESULTS

Study selection and IPD obtained

Our search identified 644 unique articles, the titles and abstracts of which were screened and ultimately 86 full-text articles were assessed for eligibility. Of these 86 articles, 11 studies [55,62-71] fulfilled the eligibility criteria (Fig 1). IPD were sought and obtained for all 11 studies, with a total of 1429 randomized participants that fulfilled the eligibility criteria. A number of the studies had additional participants that did not meet the eligibility criteria (e.g., were dark-skinned (in 5 RCTs), sampled outside the specified winter period (in 1 RCT), below the age threshold of 24 months (in 1 RCT), or had missing data on a required variable (in 4 RCTs)), and the data on these additional participants were not included in the present analysis.

Study and participant characteristics

Table 1 shows the characteristics of the 11 eligible studies and their participants. The RCTs were conducted in 8 countries within North America and Europe. Seven studies were conducted in adults, 3 in children, and 1 in both age-groups. Three studies were conducted in adult females only, the rest were conducted in studies with a mixture of males and females.

Among the 11 RCTs, mean baseline serum 25(OH)D concentrations ranged from 45.9 to 75.0 nmol/L; 2 studies had mean baseline concentrations in the range of 40–49.9 nmol/L, 4 studies each were within the range of 50–59.9 nmol/L and 60–69.9 nmol/L, respectively, and 1 study

had a mean baseline concentration >70 nmol/L (Table 1). Five of the 11 RCTs had a requirement that participants would not travel to a sunny locale and/or use tanning beds during the study. Four and 7 RCTs were conducted within latitude bands of 40 to 49.9°N and 50 to 63°N, respectively. A range of assay types were used to measure serum 25(OH)D, including RIA (in 3 studies), LC-MS/MS (in 5 studies) and one study each used HPLC, ELISA, or chemiluminescence immunoassay (Table 1).

All RCTs administered vitamin D-fortified/enhanced/enriched food(s) to participants in the intervention arms: given daily (10 RCTs) or weekly (1 RCT). The daily (or daily equivalent) dose of vitamin D provided by consumption of the assigned amount/serving size of the vitamin D-fortified/enhanced/enriched food(s) ranged from 3.5 to 100 µg/d: 5 studies used ≤10 µg/d, 1 study used 10 and 15 µg/d, 4 studies used ≥12-30 µg/d, and 1 study used 100 µg/d equivalent (Table 1). Six studies used dairy-based foods (of which 5 used a single source (cheese or a cow's milk-based beverage) and 1 used yoghurt and cheese), 1 RCT each used bread; eggs; orange juice or biscuits; or milk plus bread; or a combination of 4 foods (vitamin D-fortified low-fat cheese, yoghurt, eggs and crisp bread) (Table 1). The RCTs had a variety of consumption patterns for the study foods ranging from being consumed once per week, once per day, to participants being allowed to freely plan how they distributed the provided foods over a day or week as long as they consumed the designated amount (data not shown). Study duration ranged from 8 weeks to 6 months. A range of dietary instruments were used to assess vitamin D intake, including 72-h detailed dietary intake report (1 study), 4-day diet diary (1 study), 3-day diet records (2 studies), and semi-quantitative FFQ (7 studies; with 5 reporting their FFQ as validated for habitual vitamin D intake).

Study quality of included RCTs

All 11 studies achieved a Jadad score of ≥3 (18% and 55% with scores of 4 and 5, respectively). In terms of contributing to these scores, method of randomization was reported

in nine studies. Two studies were reported as blinded, but methods for blinding were unclear based on the information presented within the papers, upon which the Jadad scores are adjudged (this information was attained after the fact from the PIs for use in the *Risk of bias* assessment, see Supplemental information on Methods in the ‘Online Resource’). Ten of the 11 RCTs reported analytical verification of the vitamin D content of the vitamin D-fortified/enhanced/enriched food(s). All 11 studies reported data on dropouts. There was a relatively low percentage of participant dropouts (0–18.5% within a study arm) and only one study had a dropout rate of >15%. It should be noted that the Jadad scale does not assess compliance, which is an important factor in food-based interventions. Compliance rates were reported in 9 studies (range of means: 79-98% with 7 RCTs >90%), one study did not assess compliance, and another study failed to report on compliance rate even though it was assessed.

Risk of bias within studies

The summary assessments of risk of bias across domains and across the 11 RCTs are shown in **Supplemental Table 3** in ‘Online Resource’. The majority of RCTs had either a low or unclear risk of selection bias (low risk of random sequence generation and allocation concealment for 9 and 8 RCTs, respectively, and the remainder had unclear risk). In relation to performance and detection bias, a majority of RCTs (*n* 9-10) had low risk of bias for blinding of participants, personnel and of outcome assessment, with 1-2 RCTs having unclear risk in these domains. In relation to attrition bias, risk of bias in relation to incomplete outcome data was low for 10 RCTs and unclear for the remaining study. Risk of bias for selective reporting was low in all 11 RCTs. Overall, most of the information used in the present meta-regression analysis is from studies at low, or to a lesser extent, unclear risk of bias.

Rate constants of included vitamin D-fortified food RCTs versus those of vitamin D supplement RCTs

The rate constants (nmol/L per µg additional vitamin D intake) for the vitamin D-fortified

food RCTs [55,62-71] and appropriate vitamin D supplement RCTs as part of the IOM DRI exercise [4] plus a few published since [72-75], stratified by age-group and latitude band in which they were conducted, are shown in **Supplemental Table 4**. The median rate constant for all RCTs, irrespective of age, in the present IPD (excluding one RCT with the negative rate constant in older adults, see below) and the updated IOM collection were similar at 1.7 and 1.6, respectively. The median rate constant from the collection of RCTs in children (at $\geq 50^\circ\text{N}$) in the present IPD was similar (1.9) to that of the updated IOM RCT collection (2.1). For adults, the median rate constants were 2.6 and 1.9 for $\geq 50^\circ\text{N}$ RCTs in the IPD and IOM analyses, respectively; and 0.8 and 0.9 for $40\text{-}49.5^\circ\text{N}$ RCTs in the IPD and IOM analyses, respectively. There was only one RCT of older adults (>60 years) in the present IPD collection and that showed a negative rate constant (i.e., no response) [62] compared to the 1.3 nmol/L per μg additional vitamin D intake for the one RCT in the IOM collection [42]. In the present IPD, the median rate constant of RCTs in children (at $\geq 50^\circ\text{N}$) was lower (1.9) than that of RCTs in adults (2.6). In the updated IOM collection of RCTs, the median rate constant of RCTs in children (at $\geq 50^\circ\text{N}$) was similar (2.1) to that of RCTs in adults (1.9).

The IPD meta-analysis model and vitamin D DRV estimates based on the 1-stage IPD meta-analyses, including subgroup analyses

Following an assessment of the 1-stage IPD meta-analysis models, a log-log model was judged to be the best fit. The analyses included an unadjusted model (**Figure 2A**) as per IOM, NNR and SACN [4,8,9], as well as a model adjusted for covariates (mean values for baseline 25(OH)D, age and BMI) (**Figure 2B**) similar to EFSA which adjusted for baseline 25(OH)D and other covariates [7]. Based on these 1-stage IPD meta-analysis models, the estimated vitamin D intakes needed to maintain 97.5% of individuals at latitudes $\geq 40^\circ\text{N}$ and $\geq 50^\circ\text{N}$ at pre-determined serum 25(OH)D thresholds as per agencies [4,7-9], assuming minimal UVB exposure, are shown in **Table 2**. As the increase in vitamin D intake estimates moving from the 90th to the 97.5th percentile of requirements were very large, especially at the 50 nmol/L serum 25(OH)D threshold, it is important to also emphasize intake estimates at the 90th and

459 95th percentiles, as shown in **Table 3**.

460 Using the UK SACN 25(OH)D cut-off of ≥ 25 nmol/L [9], we estimated the vitamin D intake
 461 needed to maintain 97.5% of individuals above this threshold to be 5.9 $\mu\text{g/d}$ based on the
 462 unadjusted model of RCTs $\geq 40^\circ\text{N}$; this decreased to 2.0 $\mu\text{g/d}$ with adjustment for covariates
 463 (Table 2). The IOM, NNR and EFSA used 30 nmol/L to indicate an increased risk of vitamin
 464 D deficiency [4,7,8], but they did not derive a vitamin D intake for this threshold. We
 465 estimated a vitamin D intake of 12.2 $\mu\text{g/d}$ to maintain 97.5% of individuals ≥ 30 nmol/L using
 466 the unadjusted model and 4.5 $\mu\text{g/d}$ using the adjusted model. The vitamin D intake estimate
 467 allowing 97.5% of individuals to maintain serum 25(OH)D ≥ 50 nmol/L (the threshold of
 468 adequacy as selected by IOM [4], NNR [8] and EFSA [7]) was 92.3 $\mu\text{g/d}$ and 43.6 $\mu\text{g/d}$ using
 469 the unadjusted and adjusted models, respectively (Table 2). The vitamin D intake estimate
 470 required to maintain 90% and 95% of individuals ≥ 50 nmol/L was 33.4 $\mu\text{g/d}$ and 57.5 $\mu\text{g/d}$,
 471 respectively using the adjusted model, and 17.0 $\mu\text{g/d}$ and 28.1 $\mu\text{g/d}$, respectively using the
 472 unadjusted model (Table 3). Excluding the Wagner *et al.* RCT [55] data (which provided
 473 additional vitamin D as a once-weekly dose of 700 μg) from the IPD analysis led to slightly
 474 higher DRV estimates than the full RCT dataset (range: 0.1-0.5 $\mu\text{g/d}$ higher at the 25 and 30
 475 nmol/L thresholds and 2.9-4.3 $\mu\text{g/d}$ higher at the 50 nmol/L threshold, depending on the
 476 model) (data not shown).

477 By age-group, the intake estimates allowing 90%, 95% and 97.5% of children (2-17.9 y) to
 478 maintain serum 25(OH)D ≥ 50 nmol/L were two to five times lower than those of adults (≥ 18
 479 years), depending on whether they were derived from the unadjusted or adjusted model
 480 (Table 3). When the 25 or 30 nmol/L serum 25(OH)D thresholds were applied, the intake
 481 estimates at the 90th, 95th and 97.5th percentiles were also lower for children compared to
 482 adults from the unadjusted model only (Table 3). For those studies that reported compliance,
 483 there was no statistical difference ($P=0.11$) in median compliance between adults and
 484 children (at 98.2% and 95.6%, respectively).

The median requirement (i.e. EAR) at the 50 nmol/L serum 25(OH)D threshold was similar for adults and children (5.0 and 5.1 µg/d, respectively) based on the unadjusted model, but was higher for children than adults (3.8 versus 2.6 µg/d, respectively) based on the adjusted model (Table 3). Using a model which adjusted for baseline 25(OH)D only (not including BMI and age as additional covariates) showed that the EAR at 50 nmol/L was 3.6 and 2.5 µg/d for children and adults, respectively (data not shown).

Seven of the 11 RCTs from which the IPD were drawn were conducted $\geq 50^{\circ}\text{N}$, a cut-off used by the IOM [4] and NNR [8], as well as being consistent with the three RCTs used by the SACN [9]. Results of the subgroup analysis of data from these 7 RCTs in unadjusted and adjusted models conducted under the same conditions were broadly similar to the equivalent estimates from the full RCT dataset (i.e., $40\text{--}63^{\circ}\text{N}$), for all ages and for adults or children only (Table 2 for the 97.5th percentile, data not shown for the 90th and 95th percentiles).

Sensitivity analyses

A leave-one-out sensitivity analysis showed modest changes (maximum 17.6%, 16.2 µg/d) and there were no overly influential RCTs (**Supplemental Table 5** in ‘Online Resource’).

The vitamin D intake requirement estimates at the 97.5th percentile based on regression models adjusted for baseline 25(OH)D alone versus baseline 25(OH)D, age and BMI were almost identical (maximum difference between estimates, 0.4 µg/d; data not shown).

Changing the anthropometry covariate had a negligible effect on the DRV estimates.

Supplemental Table 6 in ‘Online Resource’ provides the vitamin D intake estimates for 97.5% to maintain serum 25(OH)D above the predefined thresholds based on regression models adjusted for baseline 25(OH)D, age and BMI and substituted by weight, z-scores of weights and z-scores of BMI. Using adult RCT data only, limiting the analysis to those whose BMI was less than 25 kg/m² showed that the 97.5th percentile vitamin D estimates at the 25 and 30 nmol/L thresholds were slightly lower (range 0.3–1.4 µg/d lower, depending on

the model) than those from analysis of all adults. For the 50 nmol/L threshold, shown in **Supplemental Table 7** in ‘Online Resource’, 97.5th percentile estimates were 5.2 and 14.5 µg/d lower for the adults with BMI <25 compared to the full adult dataset, using unadjusted and adjusted models, respectively

Baseline serum 25(OH)D had a major effect on estimates. Subgroup analysis, using the unadjusted regression model, showed that the vitamin D intake requirement estimates at the 97.5th percentile at each of the three serum 25(OH)D thresholds were 3- to 6-times higher in subjects whose baseline 25(OH)D concentrations was <50 nmol/L compared to those with baseline concentrations ≥50 nmol/L. At the 25 and 30 nmol/L thresholds, the respective RDA estimates (95% CI) were 11.8 (7.0, 19.8) µg/d and 23.4 (14.6, 37.0) µg/d for those with baseline 25(OH)D <50 nmol/L, and 2.1 (1.4, 3.3) µg/d and 4.8 (3.1, 7.4) µg/d for those with baseline 25(OH)D ≥50 nmol/L (data not shown). At the 50 nmol/L serum 25(OH)D threshold, the respective 90th, 95th and 97.5th percentile requirement estimates (95% CI) were 63.8 (41.2, 104.7) µg/d, 105.0 (64.5, 179.2) µg/d and 162 (94.1, 270.9) µg/d for those with baseline 25(OH)D <50 nmol/L, and 17.1 (11.6, 24.8) µg/d, 29.7 (19.7, 45.5) µg/d and 47.8 (31.3, 81.5) µg/d for those with baseline 25(OH)D ≥50 nmol/L (data not shown).

Addition of the method of vitamin D intake assessment as an additional covariate to the model (also adjusted for baseline 25(OH)D, age and BMI) yielded vitamin D estimates at the 97.5th percentile that were higher (by 0.7, 1.4 and 10 µg/d at the 25, 30 and 50 nmol/L serum 25(OH)D thresholds, respectively) than those from analysis of all adults, irrespective of intake assessment method (data not shown). In the leave-one-out sensitivity analysis, variability in the range of change in RDA estimates at the 50 nmol/L serum 25(OH)D threshold on omission of individual RCTs was evident in those which estimated vitamin D intake by FFQ (-17.6% to 22.2%) and non-FFQ assessment methods (-16.7% to 12.8%) (**Supplemental Table 4** in ‘Online Resource’).

Likewise, addition of method of serum 25(OH)D measurement as an additional covariate

within the model yielded 97.5th percentile vitamin D estimates that were higher (by 1.1, 2.2 and 16.7 µg/d at the 25, 30 and 50 nmol/L serum 25(OH)D thresholds, respectively) than those from analysis of all adults, irrespective of 25(OH)D measurement method (data not shown). In the leave-one-out sensitivity analysis, variability in the range of change in RDA estimates at the 50 nmol/L serum 25(OH)D threshold on omission of individual RCTs was evident in those which measured serum 25(OH)D by chromatographic (-17.6% to 22.2%) and non-chromatographic methods (-16.7% to 4.7%) (**Supplemental Table 4** in ‘Online Resource’).

Seven of the 11 RCTs from which the IPD were drawn had available individual-level data on compliance of consumption of the intervention food(s). An analysis of the estimates from a 1-step IPD meta-analysis model using data from this subset of 7 RCTs showed that percentage compliance with the food product had a relatively minor impact on the intake estimates at the 25 and 30 nmol/L serum 25(OH)D thresholds, i.e., estimates being 0 to 0.9 µg/d lower, depending on the intake requirement percentiles (90th to 97.5th) and whether 80% or 95% compliance threshold (**Supplemental Table 8** in ‘Online Resource’). At the 50 nmol/L serum 25(OH)D threshold, estimates were 2.5 to 10.1 µg/d lower than those from models not accounting for compliance, going from the 90th to 97.5th percentile, depending on whether 80% or 95% compliance threshold applied (Supplemental Table 7). The age-group specific differences in estimates in the full dataset from these 7 RCTs were also evident when the dataset excluded subjects with compliance <80% or <95% (Supplemental Table 7). This was also the case when unadjusted models were used (data not shown).

Safety considerations of the IPD-derived vitamin D intake requirement estimates

None of the vitamin D intake requirement estimates, derived from the adjusted models, exceeded the age-specific UL for vitamin D (Table 3). Using the intake estimates from the unadjusted models, none of the estimates for children exceeded the minimum specified UL for children (50 µg/d [35]). While the intake estimates covering 90% or 95% of adults did not, the 97.5% estimate for adults did exceed the UL (Table 3).

The projected upper 97.5th percentile serum 25(OH)D concentrations achieved at vitamin D intake requirement estimates, derived from the unadjusted and adjusted models, are shown in **Table 4**. None of the vitamin D intake requirement estimates at the 25 and 30 nmol/L serum 25(OH)D thresholds, from either the unadjusted or adjusted models, and whether covering 90, 95 or 97.5 percent of individuals, led to predicted upper 97.5th percentiles of serum 25(OH)D concentrations exceeding 150 nmol/L (maximum of 132 nmol/L at 12 µg/d intake, corresponding to the intake needed to maintain 97.5% of individuals with serum 25(OH)D $\geq$ 30 nmol/L). Using the vitamin D intake requirement estimates at the 50 nmol/L threshold, and based on the adjusted model, none of the three predicted upper 97.5th percentiles of serum 25(OH)D concentrations exceeded 200 nmol/L; with the vitamin D intakes covering 95% and 97.5% of individuals leading to 97.5th percentile serum 25(OH)D of 155 and 171 nmol/L, respectively (Table 4). Using on the unadjusted model, all three predicted upper 97.5th percentiles of serum 25(OH)D concentrations exceeded 150 nmol/L, and the vitamin D intake to cover the needs of 97.5% of individuals (92.3 µg/d) at 50 nmol/L threshold yielded a predicted upper 97.5th percentile serum 25(OH)D concentration of 220 nmol/L (Table 4). The vitamin D intakes corresponding to the EAR (50th percentile) at the 50 nmol/L threshold yielded a predicted upper 97.5th percentile serum 25(OH)D concentration of 105 and 93 nmol/L, based on unadjusted and adjusted models, respectively (data not shown).

Discussion

Since 2010, most expert agencies tasked with updating their vitamin D DRV have employed a standard meta-analysis approach based on aggregate data from RCTs, mostly with vitamin D supplements [4,7,8]. The present IPD analyses, based on pooled individual data from 11 winter-based RCTs using vitamin D-fortified foods provides new estimates for the vitamin D intakes needed to maintain individual winter-time serum 25(OH)D concentrations above commonly used thresholds. The approach used for data analysis, including the model and adjustments included, had a profound impact on the DRV estimates generated. For example, using data from RCTs conducted $\geq 50^{\circ}\text{N}$ in an unadjusted curvilinear model, we estimated that the vitamin D intakes required to maintain 97.5% of serum 25(OH)D concentrations ≥ 25 nmol/L [9] and ≥ 30 nmol/L were 6 $\mu\text{g/d}$ and 12 $\mu\text{g/d}$, respectively. Adjustment for mean values of baseline 25(OH)D, age and BMI reduced these estimates to 2.5 and 5.1 $\mu\text{g/d}$, respectively. Staying with studies $\geq 50^{\circ}\text{N}$ and switching to the 50 nmol/L threshold [4,8], the vitamin D intake required to maintain 90%, 95% and 97.5% of serum 25(OH)D concentrations ≥ 50 nmol/L was estimated to be 30, 49 and 75 $\mu\text{g/d}$, respectively. Adjustment for mean baseline 25(OH)D, age and BMI derived lower estimates of 16.1 and 25.5 $\mu\text{g/d}$ at the 90th and 95th percentiles and 38 $\mu\text{g/d}$ at the 97.5th percentile. Emphasis on the estimates at the 90th and 95th percentiles in the present work is of importance as the estimates at the 97.5th were very high and need to be considered with caution.

The striking differences between the estimates at the 90th and 95th, as well as the 97.5th, percentiles for adults at the 50 nmol/L threshold from the present food trial-based and previous supplement trial-based IPD analyses [28] and the recommended 10 $\mu\text{g/d}$ by NNR [8] and 15 $\mu\text{g/d}$ by IOM (for those aged 1-70 y) [4] and EFSA [7] relate to the fact that the standard meta-analysis, as applied by these agencies, is not able to add the two required standard deviations to the median serum 25(OH)D response to cover the 97.5th percentile of individuals, as information on the between-individual variability is not accessible [29]. The IPD approach is highly relevant and applicable in this regard as between-participant variability is crucial for estimating individual-based DRV, such as the RDA, RNI, RI, and the EU's *Population Reference Intake* (PRI) [29]. SACN's RNI of 10 $\mu\text{g/d}$ was based on

613 regression analysis of individual data, but only from one RCT each in children, adults and
614 older adults [9], unlike the present IPD which used pooled individual data from 11 RCTs.

615
616 The present vitamin D intake estimates at the 90th, 95th and 97.5th percentiles using all three
617 serum 25(OH)D thresholds (25, 30 and 50 nmol/L) from the unadjusted models were 2- to 5-
618 fold higher for adults than for children. Likewise, the 90th, 95th and 97.5th percentile
619 estimates at the 50 nmol/L serum 25(OH)D threshold from the adjusted model were ~2-times
620 higher for adults than children; even though estimates were broadly similar for adults and
621 children at the two lower thresholds. In contrast, IOM, NNR and EFSA, who combined data
622 from RCTs in children and in adults (up to 60 years in the case of NNR) in their respective
623 analyses [4,7,8], established the same vitamin D DRV for children and adults. SACN used
624 data from a RCT in 11-year-old girls as well as one RCT each in adults and older adults [9].
625 Brett *et al.* [26] in a subgroup analysis within their recent meta-analysis based on aggregate
626 data of vitamin D RCTs in children aged 2-18 y, reported the mean change in serum
627 25(OH)D per 2.5 µg vitamin D/d supplied as fortified food (9.3 nmol/L), and highlighted that
628 this was about 3-times higher than that reported in either of the two equivalent meta-analyses
629 of adults (~3 nmol/L; [21,22]). Lower requirements for vitamin D in children have been
630 suggested to be possibly linked to body size [26,72,76], with children being, on average,
631 smaller than adults and their tissue stores taking up a smaller amount of vitamin D from the
632 circulation [26]. A greater volumetric dilution of ingested vitamin D within adults compared
633 to children, is akin to that suggested to explain the lower vitamin D status among obese
634 versus healthy weight adults [77]. The 2-fold difference in vitamin D requirement estimates
635 at the 50 nmol/L serum 25(OH)D threshold between children and adults in the present IPD
636 remained even when body weight or z-scores for weight or BMI were substituted for BMI as
637 a covariate within the analyses. There may be other factors contributing to the lower
638 variability in serum 25(OH)D response to a specific vitamin D intake in children compared to
639 that seen in adults. There were no significant differences in compliance between children and
640 adults for those RCTs which had data available, suggesting this was likely not a key
641 contributory factor.

642
643 That the extra vitamin D supplied in the RCTs included in the present IPD was by means of
644 food vehicles is both timely and important as much of the analysis to-date has focussed on

data from vitamin D supplement RCTs [4,7-9]. Only EFSA included limited data from RCTs with vitamin D-fortified foods in their analyses (e.g., 18 RCT arms out of total of 83) [7]. It is worth considering that estimates arising from food-based studies may be highly relevant as the WHO–FAO [20] and others [3,14,19,25,78] have highlighted the advantages of a fortified food-based approach over supplements as a more effective public health strategy for increasing vitamin D in the food chain. While acknowledging the WHO–FAO’s suggestion that increasing dietary diversity may be the most preferred way of addressing micronutrient malnutrition [20], this is not feasible for vitamin D, because there are very few naturally rich food sources [14]. However, increasing diversity in the context of types of foods fortified with vitamin D has been emphasised [14,21,23,25,78], as a wider suite of foods, in addition to milk/other dairy-based products (such as cheese and yoghurts), has the potential to increase vitamin D intakes in the population, especially among non- or low-dairy consumers. In this regard, the overall findings of this IPD highlight how vitamin D fortification of breads, biscuits, orange juice, and eggs, as well as milk/dairy products, alone or in some combination(s), can increase vitamin D status in children and adults.

While, the response of serum 25(OH)D to increased vitamin D intake from fortified cheese (i.e., the rate constant) was unexplainably low in one RCT [62], the rate constants from the other 10 RCTs with vitamin D-fortified foods and from RCTs with vitamin D supplements were comparable, suggesting similar bioavailability across a range of food products. The present IPD’s finding that 12 µg/d of vitamin D, supplied by fortified foods together with habitual intake, can prevent wintertime vitamin D deficiency (serum 25(OH)D <30 nmol/L) in the vast majority of individuals is broadly in line with nationally representative data from Finland, who have one of the most progressive vitamin D food fortification programmes in Europe. For example, the representative National *FINDIET 2012* survey in Finland has shown that the mean daily vitamin D intake has increased from 3 and 5 µg/day for women and men, respectively, in 2002 (prior to instigation of food fortification a year later) to 18 and 17 µg/day for women and men, respectively, in 2012 [79]. Such increases in vitamin D intakes have been mirrored by notable improvements in status over the same period. Data from the *Finnish Health* surveys in 2000 and 2011 show that the mean standardized serum 25(OH)D increased from 48 nmol/L to 65 nmol/L, and prevalence of serum 25(OH)D <30 nmol/L decreased from 13% to <1% [80]. From a safety perspective, the present IPD

suggested a vitamin intake of 12 µg/day yielded an upper 97.5th percentile serum 25(OH)D of 132 nmol/L and the 2011 *Finnish Health* survey only reported 0.2% of its 4,051 participants with serum 25(OH)D >125 nmol/L [80].

Limitations of the present IPD analysis are exemplified in the variation in the 97.5th percentile estimates at the 50 nmol/L 25(OH)D threshold, much of which was attributable to less than 10% of the population (i.e., moving from the 90th to 97.5th percentile). Sensitivity analyses were used to explore the potential sources of this variability, as the 92 µg vitamin D/day required to maintain 97.5% of individuals with serum 25(OH)D >50 nmol/L, based on the unadjusted model, predicted an upper 97.5th percentile serum 25(OH)D of 220 nmol/L, which is well in excess of the UL. Sub-group analysis for RCTs where compliance data were available showed that at the 50 nmol/L serum 25(OH)D threshold, intake estimates at the 97.5th percentile were 8.4 and 10.1 µg/d lower when a minimum compliance threshold of 80% and 95%, respectively, were applied compared to estimates from the model not accounting for compliance. In terms of other potential sources of variability, there can be substantial variability associated with laboratory measurement of serum 25(OH)D [81]. The use of standardized serum 25(OH)D data has many merits in overcoming some of this method-related differences in estimates [82], but this is not always feasible, particularly for RCTs. In addition, the estimates of total vitamin D intake used in the analyses are subject to measurement errors arising from the variety of different dietary assessment techniques used by the various RCTs. To estimate habitual vitamin D intakes, a time frame of sufficient duration to capture infrequent food consumption (e.g. liver, fatty fish) is required (e.g. a month is reasonable) [82], which is why many authors used validated FFQ methods to avoid underreporting. On the other hand, it has been suggested that FFQ often tend to overestimate nutrient intakes compared to food record-based approaches [82], however this has not been verified for vitamin D. Differences in the coverage of vitamin D in foods within different food compositional databases [1,25] used to estimate vitamin D intake, as well as the vitamin D compounds included, may also have introduced variability into our analysis. Our sensitivity analyses showed that adjusting for method of vitamin D assessment and for method of serum 25(OH)D measurement increased the intake estimates at the 97.5th percentile by 0.7, 1.4 and 10 µg/d, and 1.1, 2.2 and 16.7 µg/d, respectively, at the respective

708 ≥ 25 , ≥ 30 and ≥ 50 serum 25(OH)D thresholds. In addition, the leave-one-out sensitivity
709 analysis did not highlight use of chromatographic versus non-chromatographic or FFQ versus
710 non-FFQ methods of vitamin D status and intake assessment in RCTs, respectively, as key
711 factors in driving the high RDA estimates at the 50 nmol/L threshold. While this sensitivity
712 analysis also suggested there were no overly influential RCTs, it is possible that there was a
713 conflation of the variability in compliance, intake estimates and 25(OH)D analytical data,
714 which when combined with between-individual variability in dose-response, contributed to
715 the very high DRV estimates at the 50 nmol/L threshold in the unadjusted analysis.

716 The strengths of the present work include the application of the IPD approach to the data
717 from 11 vitamin D-fortified food-based RCTs in both children and adults which met or
718 exceed the eligibility criteria of IOM and/or EFSA, and which were identified through a
719 systematic review thus increasing the external validity of our findings. Study quality was
720 generally high and the majority of data are recent, with 9 of 11 of trials published in the last 7
721 years. The majority of studies had a low risk of bias across the 7 categories.

722 In conclusion, this IPD analyses of food-based vitamin D RCTs has provided new DRV
723 estimates for vitamin D using models both adjusted and unadjusted for baseline serum
724 25(OH)D and other covariates. The approach used for data analysis, including model and
725 adjustments included, had a profound impact on the DRV estimates generated. The work
726 highlights the importance of being able to capture between-participant variability, crucial for
727 estimating individual-based DRV recommendations. The DRV estimates for the 50 nmol/L
728 25(OH)D threshold both here and from our previous supplement trial-based IPD analysis are
729 higher than the recommendations from NNR, IOM and EFSA, which used standard meta-
730 regression based on aggregate data from vitamin D supplement RCTs. The present IPD also
731 shows that the RNI and RI/RDA estimates at the 25 and 50 nmol/L serum 25(OH)D
732 threshold, respectively, for children are ≥ 2 times lower than those for adults, which differs
733 from agency reports to date. Finally, the IPD work also further informs and adds to the
734 evidence-base around implementation of food-based approaches to tackle inadequate vitamin
735 D intake and status. For example, the present findings provide evidence on how using food-
736 based approaches in attaining an intake of ~ 12 $\mu\text{g/d}$ could prevent very low vitamin D status
737 (i.e., serum 25(OH)D < 30 nmol/L), with significant potential for public health benefit.

Declarations

Authors' contributions to the manuscript

KDC, MEK and CR designed the research; KDC and MEK defined the eligibility criteria for the food-based vitamin D randomized controlled trials (RCTs) for inclusion in the individual participant data (IPD)-level meta-regression analysis; KDC conducted the electronic searches; Study selection and decision on which met the eligibility criteria and we included was conducted by KDC and MEK; KDC, LT and MEK performed the quality assessment of eligible studies; KDC, RA, IMG, KHM, JN, IT, LT, SAL-N, LT, MPV, UT, GJ, VVM, BLS, JH, AK, DW, RV, IO, PKA, NRB, HAW accessed, formatted and supplied the IPD from their 11 included RCTs which formed the core pooled dataset upon which this work was based; An assessment of the risk of bias in the included RCTs was performed by KDC and LT; CR performed the statistical analyses and derivation of vitamin D dietary reference value estimates; KDC and CR wrote the first draft of the paper, with all authors providing input and comment on subsequent versions; All authors read and approved the final manuscript.

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Code availability: The R code for fitting linear and nonlinear models is presented in Cashman KD, Ritz C (2019). Individual participant data (IPD)-level meta-analysis of randomised controlled trials among dark-skinned populations to estimate the dietary requirement for vitamin D. Syst Rev 8:128. doi: 10.1186/s13643-019-1032-6.

Compliance with ethical standards

Ethics standards

Approval by a research ethics committee to conduct this meta-analysis was not required because the aim of this secondary analysis was consistent with the ethical approval received for the individual studies. The current analysis was conducted on anonymized data.

Conflicts of Interest:

The following authors had no conflicts of interest: Kevin D. Cashman, Mairead E. Kiely, Rikke Andersen, Ida M. Grønberg, Katja H. Madsen, Janna Nissen, Inge Tetens, Laura Tripkovic, Laura Toxqui, M. Pilar Vaquero, Ulrike Trautvetter, Gerhard Jahreis, Vikram V. Mistry, Bonny L. Specker, Jürgen Hower, Anette Knoll, Dennis Wagner, Reinhold Vieth, Inger Öhlund, Pia Karlsland Åkeson, Neil R. Brett, Christian Ritz.

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773 Hope A. Weiler at the time of the trial received a small speaker's honoraria for presenting at
774 the annual conferences organized by Dairy Farmers of Canada [Weiler H. Role of dairy in
775 body composition and health. Dairy Research Symposium, Dairy Farmers of Canada and
776 Agriculture Canada, Ottawa, Feb 5, 2016].

777

778 Susan A. Lanham-New is Research Director of D3Tex Ltd which holds the UK and Gulf
779 Corporation Council (GCC) Patents for the use of UVB material in preventing vitamin D
780 deficiency in women who dress for cultural style. SLN also received a small speaker's
781 honoraria for presentations at two conferences on Vitamin D organised by Solaris.

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Table 1. Selected design parameters of the 11 randomized controlled trials with vitamin D-fortified food, conducted in winter and $\geq 40^\circ\text{N}$, as well as baseline and vitamin D-related outcome characteristics of white subjects who completed the intervention studies and were included in data analyses*.

Study	Johnson <i>et al.</i> 2005	Wagner <i>et al.</i> 2008	Madsen <i>et al.</i> 2013	Toxqui <i>et al.</i> 2014
(Reference Number)	[62]	[57]	[64]	[65]
Trial registry ID	NR	NR	NCT01184716	NCT01739907
Design parameters:				
Location ($^\circ\text{N}$)	USA ($\sim 44^\circ\text{N}$)	Canada (43°N)	Denmark (56°N)	Spain (40.2°N)
Year of study	2004	2007	2010-2011	2012
Duration (and Months)	2 months (within Dec–April)	8 weeks (within Jan–April)	6 months (Sept–April)	8 weeks (within January–March)**
Food interventions [supplemental vitamin D ₃ dose]	Vit D-fortified cheese [15 $\mu\text{g}/\text{d}$] <i>v.</i> Non-fortified cheese <i>v.</i> No cheese	Fortified [700 $\mu\text{g}/\text{wk}$] <i>v.</i> Low fat-fortified [700 $\mu\text{g}/\text{wk}$] <i>v.</i> Placebo cheese	Vit D-fortified 0.5% milk + bread (wheat/rye) [6.8 $\mu\text{g}/\text{d}$, on average] <i>v.</i> Non-fortified milk and bread	Fe and Vit D-fortified skimmed cow's milk [5 $\mu\text{g}/\text{d}$] <i>v.</i> Fe-fortified skimmed cow's milk
Subject characteristics:				
<u>Baseline</u>				
<i>N</i>	90	50	682	107
Sex (Male:Female)	34:56	25:25	330:352	0:107
Age (year)	73.5 ± 7.2^1	27.8 ± 9.6	27.2 ± 16.3	24.9 ± 4.4
Weight (kg)	79.8 ± 15.8	68.2 ± 14.6	60.6 ± 24.8	59.5 ± 8.9
BMI (kg/m^2)	28.3 ± 5.2	23.8 ± 4.2	21.9 ± 5.4	21.7 ± 3.0
Dietary vitamin D ($\mu\text{g}/\text{day}$) ²	11.1 ± 7.8^d	3.2 ± 2.3^a	2.8 ± 1.7^a	2.9 ± 2.8^b
Serum 25(OH)D (nmol/L) ³	51.8 ± 19.3^e	53.8 ± 21.4^e	74.8 ± 19.9^f	61.7 ± 19.6^i
<u>Endpoint</u>				
Dietary vitamin D ($\mu\text{g}/\text{day}$) ⁴ :				
Control group(s) (<i>n</i>)	10.9 ± 8.1 (61)	3.9 ± 3.0 (20)	4.2 ± 3.8 (353)	2.9 ± 2.5 (52)
Vit D intervention group(s) (<i>n</i>)	26.0 ± 7.1 (29)	103.9 ± 3.3 (30)	13.5 ± 6.9 (329)	7.3 ± 3.3 (55)
Serum 25(OH)D (nmol/L) ^{3,4} :				
Control group(s) (<i>n</i>)	51.0 ± 16.3 (61)	50.7 ± 24.2 (20)	45.1 ± 20.2 (353)	58.1 ± 17.5 (52)
Vit D intervention group(s) (<i>n</i>)	53.2 ± 19.9 (29)	120.0 ± 28.2 (30)	70.0 ± 19.0 (329)	65.7 ± 16.6 (55)

Study	Trautvetter <i>et al.</i> 2014	Hayes <i>et al.</i> 2016	Tripkovic <i>et al.</i> 2017	Grønborg <i>et al.</i> 2019
(Reference Number)	[66]	[68]	[70]	[71]
Trial registry ID	NCT01297023	NCT02678364	ISRCTN23421591	NCT02631629
Design parameters:				
Location (°N)	Germany (51°N)	Ireland (51.9°N)	UK (51.3°N)	Denmark (56°N)
Year of study	2011	2015	2011-2012 and 2012-2013	2016
Duration (and Months)	8 weeks (within Jan–April)	8 weeks (within Jan–March)	12 weeks (within Oct–March)	3 month (Jan–March)
Food interventions [supplemental vitamin D ₃ dose]	CaP <i>v.</i> Vit D <i>v.</i> CaP + Vit D [10 µg/d] -fortified bread	Vit D [3.5 µg/d]- <i>v.</i> 25-(OH)D [4.5 µg/d]- fortified eggs <i>v.</i> Control eggs	Vit D ₃ -fortified Orange Juice [15 µg/d] <i>v.</i> Vit D ₃ -fortified Biscuit [15 µg/d] <i>v.</i> Placebo	Vitamin D-fortified low-fat cheese, yoghurt, eggs and crisp bread (supplying 30 µg/d in total) <i>v.</i> non- fortified equivalents
Subject characteristics:				
<u>Baseline</u>				
N	55	50	133	60
Sex (Male:Female)	23:32	26:24	0:133	0:60
Age (year)	42.8 ± 12.1	54.9 ± 6.4	46.2 ± 12.1	33.3 ± 11.3
Weight (kg)	72.9 ± 14.3	73.7 ± 15.1	65.5 ± 10.8	67.8 ± 12.0
BMI (kg/m ²)	24.8 ± 3.9	25.4 ± 4.1	23.8 ± 3.5	24.1 ± 4.1
Dietary vitamin D (µg/day) ²	3.6 ± 4.1 ^d	6.6 ± 3.8 ^a	2.9 ± 2.4 ^c	1.5 ± 0.8 ^a
Serum 25(OH)D (nmol/L) ³	50.8 ± 21.8 ^h	45.9 ± 16.4 ^f	60.2 ± 24.7 ^f	48.6 ± 16.3 ^f
<u>Endpoint</u>				
Dietary vitamin D (µg/day) ⁴ :				
Control group(s) (n)	6.6 ± 0.5 (18)	6.0 ± 2.7 (16)	2.5 ± 2.3 (43)	1.5 ± 0.7 (31)
Vit D intervention group(s) (n)	15.0 ± 0.8 (37)	10.0 ± 3.8 (34)	8.1 ± 2.5 (90)	28.7 ± 8.8 (29)
Serum 25(OH)D (nmol/L) ^{3,4} :				
Control group(s) (n)	47.4 ± 30.3 (18)	35.1 ± 11.0 (16)	44.1 ± 17.8 (43)	44.0 ± 16.7 (31)
Vit D intervention group(s) (n)	67.7 ± 13.9 (37)	48.5 ± 18.7 (34)	90.7 ± 24.0 (90)	77.8 ± 14.4 (29)

Study	Hower <i>et al.</i> 2013	Brett <i>et al.</i> 2016	Öhlund <i>et al.</i> 2017
(Reference Number)	[63]	[67]	[69]
Trial registry ID	NR	NCT02097160	NCT01741324
Design parameters:			
Location (°N)	Germany (51°N)	Canada (45.5°N)	Sweden (55 and 63°N)
Year of study	2010-2011	2014	2012–2013
Duration (and Months)	4 months (Nov–Feb)**	12 weeks (Jan–April)	3 months (within Nov–March)
Food interventions [supplemental vitamin D ₃ dose]	Vit D-fortified growing up milk [10 µg/d] <i>v.</i> Semi-skimmed cow's milk with no added vitamin D	Vit D-fortified drinkable yogurt + cheese (providing 10 or 15 µg/d) <i>v.</i> non-fortified yogurt + cheese	Vit D-fortified lactose-free, UHT milk [12 or 22 µg/d] <i>v.</i> non-fortified lactose-free, UHT milk
Subject characteristics:			
<u>Baseline</u>			
<i>n</i>	62	43	97
Sex (Male:Female)	32:30	22:21	45:52
Age (year)	3.7 ± 1.2	5.5 ± 1.8	6.3 ± 0.6
Weight (kg)	16.3 ± 3.4	21.4 ± 5.9	23.1 ± 3.6
BMI (kg/m ²) [z score]	15.9 ± 1.5 [0.16 ± 1.0]	16.4 ± 1.4 [0.43 ± 0.81]	15.8 ± 1.5 [0.04 ± 1.0]
Dietary vitamin D (µg/day) ²	2.2 ± 1.4 ^a	5.2 ± 2.6 ^a	5.5 ± 2.7 ^a
Serum 25(OH)D (nmol/L) ³	54.7 ± 18.8 ⁱ	60.9 ± 12.5 ^e	63.9 ± 16.9 ^f
<u>Endpoint</u>			
Dietary vitamin D (µg/day) ⁴ :			
Control group(s) (<i>n</i>)	2.5 ± 1.5 (23)	6.2 ± 1.8 (15)	6.3 ± 2.4 (18)
Vit D intervention group(s) (<i>n</i>)	8.7 ± 2.8 (39)	12.8 ± 3.8 (28)	22.4 ± 6.0 (79)
Serum 25(OH)D (nmol/L) ^{3,4} :			
Control group(s) (<i>n</i>)	37.4 ± 16.6 (23)	58.6 ± 13.1 (15)	59.2 ± 14.5 (18)
Vit D intervention group(s) (<i>n</i>)	64.2 ± 22.9 (39)	63.6 ± 12.0 (28)	80.5 ± 16.6 (79)

*In some cases, additional participants in the study were not included in the analyses as they did not meet with the inclusion criteria (total *n* = 489).

**Not study endpoint but sampling point which fit with sampling months as specified by inclusion criteria. ¹Mean ± SD (all such values).

²Habitual dietary vitamin D intake assessed via semi-quantitative FFQ^a, 72-h detailed dietary intake report^b, 4-day diet diary^c, or 3-day diet records^d.

³Serum 25(OH)D measured by RIA^e, LC-MS/MS^f, HPLC^g, ELISA^h, or chemiluminescence immunoassayⁱ.

⁴In studies where there were more than one control and/or vitamin D intervention groups, reported values are for all control or vitamin D intervention subjects.

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; NR, not registered.

Table 2. Individual Participant Data (IPD) meta-analysis-derived dietary requirements for vitamin D ($\mu\text{g/day}$) for allowing 97.5% of individuals to maintain serum 25(OH)D at or above three recommended thresholds¹.

	Model 1	Model 2	Model 3	Model 4
Serum 25(OH)D²	$\geq 40^\circ\text{N}$; No adjustments ³	$\geq 40^\circ\text{N}$; Adjusted ⁴	$\geq 50^\circ\text{N}$; No adjustments ⁵	$\geq 50^\circ\text{N}$; Adjusted ⁶
All	<i>n</i> =1429	<i>n</i> =1429	<i>n</i> =1139	<i>n</i> =1139
≥ 25 nmol/L	5.9 (3.9, 9.0)	2.0 (1.3, 3.4)	6.3 (4.0, 9.7)	2.5 (1.5, 4.3)
≥ 30 nmol/L	12.2 (8.2, 18.4)	4.5 (2.8, 7.5)	12.1 (7.9, 19.2)	5.1 (3.1, 8.5)
≥ 50 nmol/L	92.3 (60.3, 146.0)	43.6 (27.7, 70.5)	75.1 (48.1, 123.5)	37.9 (23.3, 69.7)
Adults (<i>aged 18-89</i>)	<i>n</i> =911	<i>n</i> =911	<i>n</i> =911	<i>n</i> =665
≥ 25 nmol/L	8.4 (4.2, 18.2)	2.2 (1.0, 4.4)	9.6 (4.7, 21.4)	3.0 (1.3, 7.5)
≥ 30 nmol/L	18.0 (9.5, 39.7)	5.2 (2.7, 10.9)	18.9 (9.4, 42.7)	6.4 (2.8, 15.3)
≥ 50 nmol/L	156.4 (76.7, 280.8)	60.2 (31.2, 120.4)	127.9 (63.9, 271.0)	52.0 (23.6, 135.9)
Children (<i>aged 2-17</i>)	<i>n</i> =518	<i>n</i> =518	<i>n</i> =518	<i>n</i> =474
≥ 25 nmol/L	3.4 (2.9, 4.0)	1.9 (1.3, 2.9)	3.6 (3.0, 4.2)	2.1 (1.3, 3.7)
≥ 30 nmol/L	6.3 (5.3, 7.2)	3.7 (2.5, 5.6)	6.5 (5.6, 7.6)	4.1 (2.5, 7.0)
≥ 50 nmol/L	33.8 (28.5, 42.0)	24.4 (16.8, 38.2)	34.3 (28.4, 43.3)	26.1 (16.4, 45.4)

¹Based on a 1-stage IPD log-log model using data from RCTs performed during winter (at either $\geq 40^\circ\text{N}$ or $\geq 50^\circ\text{N}$) and which related serum 25(OH)D concentration as a function of vitamin D intake (with or without adjustment). 95% CIs for the lower prediction limits were obtained using bias-corrected bootstrap based on 1000 replications.

²Cut-off of ≥ 25 nmol/L used by UK Scientific Advisory Committee on Nutrition [22]; cut-off of ≥ 30 nmol/L used by Institute of Medicine [IOM] [4], Nordic Nutrition Recommendations [NNR] [21] and European Food Safety Authority [EFSA] [22] to define deficiency, and cut-off of ≥ 50 nmol/L used by IOM, NNR and EFSA to define threshold of adequacy.

³Model 1: RCTs performed in the latitude band of $40-63^\circ\text{N}$; no adjustments to model.

⁴Model 2: RCTs performed in the latitude band of $40-63^\circ\text{N}$; adjustment in model for baseline serum 25(OH)D, age and BMI.

⁵Model 3: RCTs performed in the latitude band of $51-63^\circ\text{N}$; no adjustments to model.

⁶Model 4: RCTs performed in the latitude band of $51-63^\circ\text{N}$ adjustment in model for baseline serum 25(OH)D, age and BMI.

Table 3. Individual Participant Data (IPD) meta-analysis-derived dietary requirements for vitamin D ($\mu\text{g/day}$) to maintain stated percentage of the population (percentile) at a serum 25(OH)D level at or above selected concentrations in participants residing between 40-63°N during winter¹

Serum 25(OH)D	50 th Percentile ²	90 th Percentile	95 th Percentile	97.5 th Percentile ³
<i>No adjustments</i>				
All (n=1429)				
≥25 nmol/L	-	2.2 (1.4, 3.2)	3.7 (2.5, 5.7)	5.9 (3.9, 9.0)
≥30 nmol/L	-	4.4 (3.0, 6.5)	7.6 (5.1, 10.8)	12.2 (8.2, 18.4)
≥50 nmol/L	4.9 (3.5, 7.0)	33.4 (23.0, 50.5)	57.5 (38.8, 87.3)	92.3 (60.3, 146.0)
Adults (n=991)				
≥25 nmol/L	-	2.6 (1.3, 4.7)	4.8 (2.4, 9.5)	8.4 (4.2, 18.2)
≥30 nmol/L	-	5.5 (3.0, 9.9)	10.4 (5.4, 20.7)	18.0 (9.5, 39.7)
≥50 nmol/L	5.0 (2.8, 8.4)	47.2 (25.3, 91.4)	89.6 (46.7, 183.7)	156.4 (76.7, 280.8)
Children (n=518)				
≥25 nmol/L	-	1.8 (1.5, 2.1)	2.5 (2.1, 3.0)	3.4 (2.9, 4.0)
≥30 nmol/L	-	3.3 (2.8, 3.8)	4.6 (4.0, 5.3)	6.3 (5.3, 7.2)
≥50 nmol/L	5.1 (4.6, 5.6)	17.5 (15.3, 20.1)	24.9 (21.2, 29.7)	33.8 (28.5, 42.0)
<i>Adjusted model</i>				
All (n=1429)				
≥25 nmol/L	-	0.8 (0.5, 1.2)	1.3 (0.8, 2.1)	2.0 (1.3, 3.4)
≥30 nmol/L	-	1.8 (1.1, 2.8)	2.9 (1.9, 4.5)	4.5 (2.8, 7.5)
≥50 nmol/L	2.9 (1.9, 4.1)	17.0 (11.3, 27.0)	28.1 (18.1, 45.9)	43.6 (27.7, 70.5)
Adults (n=911)				
≥25 nmol/L	-	0.7 (0.4, 1.4)	1.3 (0.6, 2.4)	2.2 (1.0, 4.4)
≥30 nmol/L	-	1.8 (0.9, 3.2)	3.1 (1.6, 6.3)	5.2 (2.7, 10.9)
≥50 nmol/L	2.6 (1.4, 4.5)	20.2 (10.9, 39.6)	36.2 (19.8, 76.0)	60.2 (31.2, 120.4)
Children (n=518)				
≥25 nmol/L	-	1.0 (0.7, 1.4)	1.4 (0.9, 2.1)	1.9 (1.3, 2.9)
≥30 nmol/L	-	2.0 (1.4, 2.7)	2.8 (1.9, 4.0)	3.7 (2.5, 5.6)
≥50 nmol/L	3.8 (2.7, 5.1)	12.8 (9.1, 17.7)	18.0 (12.3, 27.1)	24.4 (16.8, 38.2)

¹Results based on a 1-stage IPD log-log model which related serum 25(OH)D concentration as a function of vitamin D₃ intake both unadjusted and adjusted for baseline serum 25(OH)D (mean), age (mean) and BMI (mean). 95% CIs for the lower prediction limits were obtained using bias-corrected bootstrap based on 1000 replications.

²The vitamin D intake that will maintain serum 25(OH)D concentrations in 50% of individuals above 50 nmol/L during winter, representing an EAR at that threshold; this is not appropriate to do at the 25 and 30 nmol/L thresholds.

³The vitamin D₃ intake that will maintain serum 25(OH)D concentrations in 97.5% of individuals above the indicated cut-off concentration during winter, representing a Recommended dietary allowance (RDA).

Table 4. Projected upper 97.5th percentile serum 25(OH)D concentrations achieved at vitamin D intake requirement estimates from the unadjusted and adjusted models¹

Requirement percentile (at specified threshold)	Vitamin D intake requirement (µg/d) ²	Projected upper 97.5 th percentile serum 25(OH)D concentrations (nmol/L)
<i>Unadjusted model</i>		
≥25 nmol/L:		
90 th Percentile	2.2	85.4
95 th Percentile	3.7	97.4
97.5 th Percentile	5.9	109.6
≥30 nmol/L:		
90 th Percentile	4.4	101.8
95 th Percentile	7.6	116.9
97.5 th Percentile	12.2	131.8
≥50 nmol/L:		
90 th Percentile	33.4	170.1
95 th Percentile	57.5	195.2
97.5 th Percentile	92.3	220.2
<i>Adjusted model</i>		
≥25 nmol/L:		
90 th Percentile	0.8	69.4
95 th Percentile	1.3	77.4
97.5 th Percentile	2.0	85.3
≥30 nmol/L:		
90 th Percentile	1.8	83.3
95 th Percentile	2.9	92.8
97.5 th Percentile	4.5	102.5
≥50 nmol/L:		
90 th Percentile	17.0	138.4
95 th Percentile	28.1	155.1
97.5 th Percentile	43.6	171.3

¹Projected upper 97.5th percentile serum 25(OH)D results based on the 1-stage IPD log-log models which related serum 25(OH)D concentration as a function of vitamin D₃ intake both unadjusted and adjusted for baseline serum 25(OH)D (mean), age (mean) and BMI (mean).

²Vitamin D intake estimates as per Tables 3.

Legends for Figures

Figure 1. PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*) flow diagram for study selection procedure.

*ClinicalTrials.gov, Cochrane Central Register of Controlled Trials, and the International Standard Randomized Controlled Trials Number registries.

Figure 2. The relation between serum 25-hydroxyvitamin D (25(OH)D) concentrations (in extended winter) and total vitamin D intake in healthy individuals aged 2-89 years living between 40°N and 63°N based on individual participant data (IPD) ($n=1429$ individuals). The solid central diagonal lines correspond to the fitted regression lines based on one-stage IPD meta-analysis (unadjusted model (Panel A) and model adjusted for age, BMI and baseline 25(OH)D (Panel B)) and the corresponding 95% prediction bands are shown in gray. *Note:* the fitted curve and the 95% confidence band were back-transformed but are displayed in the graphs using logarithmic axes in keeping with the log-log model. The black horizontal lines in each panel represent the serum 25(OH)D thresholds of 30 and 50 nmol/L relating to risk of vitamin D deficiency and inadequacy, respectively. Overlapping dots make some appear more darkly coloured.

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