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The relationship between vitamin D intake and serum 25-hydroxyvitamin D in young children: a meta-regression to inform WHO/FAO vitamin D intake recommendations

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Abbreviations:

25OHD: 25-hydroxyvitamin D
CI: Confidence intervals
CV: Coefficient of variation
EIA: Enzyme immunoassay
FAO: Food and Agriculture Organisation of the United Nations
GDD: Global Dietary Database
GRADE: Grades of Recommendation, Assessment, Development, and Evaluation
HPLC: High-performance liquid chromatography
INL98: Individual Nutrient Level 98
LC-MS/MS: Liquid chromatography with tandem mass spectrometry
NOAEL: No observed adverse effect level
NR: not reported
RCTs: Randomized controlled trials
RIA: Radioimmunoassay
UL: Upper level
WHO: World Health Organisation

Abstract

Background: This work was commissioned by the WHO and FAO to inform their update of the vitamin D requirements for children below 4 years old.

Objective: The objective of this work was to undertake multi-level and multivariable dose-response modelling of serum 25OHD to total vitamin D intake in children below 4 years of age and to derive updated vitamin D requirements for young children.

Methods: Systematically identified randomized controlled trials among healthy children from 2 weeks up to 3.9 years of age provided with daily vitamin D supplements or vitamin D-fortified foods were included. Linear and non-linear random effects multi-level meta-regression models with and without covariates were fitted and compared. Inter-individual variability was included by simulating the individual serum 25OHD responses. The percentage of individuals reaching set minimal and maximal serum 25OHD thresholds were calculated and used to derive vitamin D requirements.

Results: A total of 31 trials with 186 data points, from North America, Europe, Asia and Australasia/Oceania, with latitudes ranging from 38°S to 61°N, and with participants of likely mostly light or medium skin pigmentation, were included; in 29 studies the children received vitamin D supplements and in two studies the children received vitamin D fortified milks with or without supplements. The dose-response relationship between vitamin D intake and serum 25OHD was best fitted with the unadjusted quadratic model; adding additional covariates, such as age, did not significantly improve the model. At a vitamin D intake of 10 µg/d, 97.3% of the individuals were predicted to achieve a minimal serum 25OHD threshold of 28 nmol/L. At a vitamin D intake of 35 µg/d, 1.4% of the individuals predicted to reach a maximal serum 25OHD threshold of 200 nmol/L.

Conclusions: In conclusion, this paper details the methodological steps taken to derive vitamin D requirements in children below 4 years of age, including the addition of an inter-individual variability component.

Statement of Significance: We undertook a multi-level and multivariable dose-response modelling of serum 25OHD to total vitamin D intake in children below 4 years of age, integrating inter-individual variability, with the goal of deriving updated vitamin D requirements in young children worldwide. We found that a vitamin D intake of 10 µg/d is recommended to ensure that more than 97% of the children maintained their serum 25OHD >28 nmol/L and that a maximal vitamin D intake of 35 µg/d is recommended to avoid risks in 98% of the children. These findings can be used to update the vitamin D requirements in infants and young children below 4 years of age, with adjustment to local population and context.

Keywords: Serum 25-hydroxyvitamin D; children; 25OHD; meta-regression; meta-analysis; vitamin D intake; nutrient requirements

Introduction

In 2004, the World Health Organisation (WHO) and Food and Agriculture Organisation of the United Nations (FAO) published global nutrient intake requirements (1). Many countries adopt these estimates as part of their national dietary allowances and/or food standards, as well as a foundation to develop food-based dietary guidelines (2). Dietary recommendations to meet nutrient requirements are by their nature intended to be iterative, and their revision is usually based on an extended body of evidence (3). In keeping with this ethos, in 2019, the FAO-WHO decided to update their nutrient intake recommendations for infants and young children (0-3.9 years) (4), and prioritizing, among other nutrients, vitamin D, in light of the new evidence that has emerged since 2004.

The availability of a large body of new data around vitamin D has also been the stimulus for a number of other authorities to update their vitamin D recommendations in recent years (5–11). The approach followed by many of these authorities consisted of the undertaking of a sequence of independent systematic evidence-based reviews, followed by an appraisal of the evidence around the relationship of serum 25-hydroxyvitamin D (25OHD) and the determination of reference level of the critical indicator health outcome of nutrient adequacy, so as to derive population serum 25OHD targets, and these, in turn, are used to establish the recommended vitamin D intake, as overviewed elsewhere (3). To date, these vitamin D requirement exercises have had a regional focus, either North America (5), Europe-wide (7), the UK (9), or the Nordic region (6), and in conditions of minimal UVB sunlight.

In an effort to provide global vitamin D requirements, the FAO-WHO has decided to update their vitamin D intake requirements with evidence from all regions of the world and irrespective of sunlight exposure. Accounting for sunlight exposure when setting vitamin D intake requirements is very challenging for a number of reasons, not least because often it is not possible to quantify the contribution sunlight exposure makes to serum 25OHD

concentrations within the general population (9). A number of systematic reviews were commissioned to enable this update. One review highlighted serum 25OHD as a useful biomarker for vitamin D status in young children (12). Two reviews on breast milk vitamin D content (13) and breast milk intake volume (14) provided new intake exposure data. Another review proposed a definition of serum 25OHD threshold for the minimization of nutritional rickets in young children (15). An additional review provided a summary of the evidence around vitamin D intake, 25OHD status and health outcomes in young children, but included only a cursory analysis of the vitamin D dose-response relationship (16).

The objective of this work was to undertake detailed, multi-level and multivariable modelling of the response of serum 25OHD to total vitamin D intake in children below 4 years of age including inter-individual variability, in order to derive updated vitamin D requirements for young children.

Methods

Eligibility criteria

The study inclusion and exclusion criteria are summarized in **Table 1**. Studies with healthy children below 4 years of age were included, whereas those of children with diseases (e.g., rickets) and certain conditions (such as prematurity, low birth weight) were excluded. Studies with daily vitamin D supplementation or vitamin D-fortified foods were included, whereas those with weekly, monthly or single (bolus) vitamin D dose(s) were excluded. Study arms in which lactating mothers received up to 12.5 µg/d vitamin D supplements were included as well as studies with supplementation of other nutrients (e.g. calcium) concomitantly as long as the effect of vitamin D could be isolated. Only the data points from 2 weeks of age onwards were included, as vitamin D status during the first 2 weeks of life was considered more reflective of the mother's vitamin D intake rather than of the infants'. Studies with a minimum follow-up

of 2 weeks were included, as this was considered to be the minimum duration required for the vitamin D intervention to have an effect on serum 25OHD.

Study selection

Studies from a previous systematic review (16) were used as a starting point for collection of the vitamin D intake-status modelling in the present work. In brief, the latter review (PROSPERO registration number: CRD42020198843) searched online databases (Medline, Embase and Cochrane Central) from inception up to June 2020 and a total of 51 vitamin D randomized controlled trials (RCTs) were identified (16). These RCTs were screened again according to a refined set of eligibility criteria (see **Table 1**), which had been more tailored to this vitamin D requirement modelling exercise. The control groups within eligible RCTs could consist of a placebo, no vitamin D addition, or low dose vitamin D supplementation (versus a higher dose).

In addition, vitamin D guidelines and reviews from other authoritative bodies (5,7,9,17–19) were reviewed and studies not previously identified were screened against the same eligibility criteria and if eligible, included.

Data extraction

Only aggregated data were available, with the achieved serum 25OHD [nmol/L] and the vitamin intake [µg/d] both expressed as continuous variables (study arm group means). The data from the eligible RCTs (n= 26) in the original review (16), which were extracted by one investigator and spot-checked by a second investigator, were additionally verified, edited and extra information added (e.g. adding missing data points, intermediate timepoints and values reported only in figures using PlotDigitizer (plotdigitizer.sourceforge.net)). Data from newly identified studies (n=5) were also extracted by one reviewer and all of this newly extracted data was checked by another reviewer. The age of the infants was defined as their age at the corresponding time of the measurement. Data from baseline, intermediate and final timepoints,

at ages below 4 years, were extracted (Note: Within-study correlation among time-points and dose groups was accounted in the model). Standard errors which could not be derived from standard deviations, confidence intervals (CI), interquartile ranges or ranges, were imputed with the weighted mean standard error of all the included studies (20).

Total vitamin D intake was calculated as the sum of vitamin D intake from the background diet and the vitamin D intake from the supplements or fortified food interventions. Vitamin D intake from the background diet was extracted from the papers, whenever reported, or requested of the study authors by e-mail where not presented; in cases where the data was not presented nor provided by authors, it was imputed using single imputation method with data from nationally representative samples from the same country, cognate studies (i.e. same country, age, year, and feeding type), or the Global Dietary Database (GDD) (www.globaldietarydatabase.org), under the assumption that the surrogate data are sufficiently similar to the study population in terms of characteristics relevant for the dietary consumption (e.g. age, feeding type). For exclusively breastfed infants aged 0-6 months, vitamin D intakes provided by breast milk were estimated using the FAO-WHO-commissioned systematic reviews (13,14).

Risk of bias assessment

The quality of the included studies were assessed with the Cochrane Risk of Bias Tool 2.0 (21). The overall strength of the evidence was assessed with Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach (22).

Data modelling

Under the assumption that the causal relationship between vitamin D intake and risk of rickets or other adverse effects was exclusively mediated by 25OHD with no other direct relationships, a model was established integrating a number of components (illustrated in **Figure 1**). Based on this model, the daily total vitamin D intake that will maintain serum 25OHD concentrations

above or below target 25OHD thresholds in a stated percentage of individuals as is the convention with nutrient requirement recommendations (3) was estimated. Data analyses were conducted with R (v 3.6.3) and RAnalyticFlow (v 3.1.8) using the package metafor.

Unadjusted random effects multi-level meta-regression models

The relationship between doses of total vitamin D intake and study mean levels of serum 25OHD was fit using the collection of RCT data from included studies (**Figure 1A**). Random effects multi-level meta-regression models with study, study arm and time of measurements included as nested random factors to reflect the hierarchical structure in the data were used (**Figure 1B**). A continuous-time autoregressive structure was assumed for the variance under the assumption that measurements closer in time have a stronger correlation and data are not equally spaced in time. Different shapes were tested including linear, quadratic, cubic, logarithmic and 3 knots restricted cubic spline (23). The best fitting model was selected based on Akaike Information Criterion (AIC), the significance of the parameters for the dose and additional considerations related to the biological explicability of the model. The model was used to predict the mean of the serum 25OHD at different levels of vitamin intake, its 95% confidence interval (95%CI) and prediction interval (95%PI) (24).

Adjusted random effects multi-level meta-regression models

The impact of inclusion of potential covariates in the vitamin D intake-status relationship was tested with adjusted models, using backward and forward stepwise selection approaches. The infants' age, baseline 25OHD, region, country income category (according to 2020 United Nations classification), 25OHD assay, season, skin pigmentation, and latitude were considered as possible modifiers. In the absence of more appropriate data on exposure to UVB, and in order to crudely cluster participants based differences in the potential for the synthesis of vitamin D in skin, a cutaneous synthesis score with latitude and season was computed (high: ≤40°N/S or >40°N/S in Summer or Autumn, or low: >40° N/S in Winter or Spring). Sensitivity

analyses were conducted excluding where study authors categorized their participants as 'vitamin D deficient' at baseline (only one study mentioned including children with vitamin D deficiency, which was defined in that study as having a serum 25OHD concentration <50 nmol/L (25)), imputed background vitamin D intake, no external standards for vitamin D assay, use of vitamin D_2 supplements, non-exclusively breastfed infants aged 0-6 months, infants below 6 months, and follow-up of only two weeks.

Target serum 25OHD thresholds for derivation of INL98 and UL intake recommendations

Target serum 25OHD thresholds, as they relate to FAO-WHO's Individual Nutrient Level 98 (INL98) and upper level (UL) reference intakes (26), were used for the present modelling (**Figure 1C**). The INL98 is intended to estimate the total vitamin D intake needed to maintain 97.5% of individuals over a stated serum 25OHD threshold concentration (26) and was derived from the serum 25OHD threshold of 28 nmol/L for children aged 0-3 years (15). The UL is intended to estimate the total vitamin D intake which is judged to be unlikely to lead to serum 25OHD concentrations associated with adverse health effects in young children and was derived from the serum 25OHD threshold of 200 nmol/L. This threshold was identified (as a NOAEL, no observed adverse effect level) by the FAO/WHO expert group on nutrient requirements based on a systematic review of studies investigating the association between serum 25OHD and vitamin D supplementation and adverse effects, especially hypercalcemia and hypercalciuria ; full details of which will be available in the FAO/WHO report (personal communication from Dr. Jason Montez, WHO).

Integration of inter-individual variability in the modelling

As the model was only able to predict the mean group-level serum 25OHD response, the individual-level response was simulated by adding another layer to the model. The meta-regressive dose-response models on aggregate data described in the previous section provides predicted group-mean, 95%CI and 95%PI values of serum 25OHD. None of these estimators

are indicative of the serum 25OHD level achieved by an individual, as befitting the INL98 and UL definition. In order to simulate individual-level responses, the inter-individual variability was built in the model, based on a method developed by EFSA (see Section 3.5.2.4 of the EFSA vitamin D UL opinion (27)).

The inter-individual variability distribution of serum 25OHD was simulated based on the studies included in the meta-regression model as well as based on individual data collected on standardised serum 25OHD concentrations in young children (28). The inter-individual distribution was considered being left-truncated normal (minimum 0) (28), with coefficient of variation (CV) of 0.34 (weighted mean CV of the studies included) and 0.10 right-skewness (28) (**Figure 1D**) under the assumption that the shape and skewness observed in the individual data study was representative of the studies in the meta-regression. For each level of vitamin D intake between 1 and 60 µg/d, 100,000 random samples of individual serum 25OHD responses were generated using Markov Chain Monte Carlo algorithm (29), with the first 10,000 simulations discarded (burn-in step). The mean of each inter-individual distribution at each vitamin D intake level was set as the predicted mean 25OHD from the best-fitting meta-regression dose-response model for deriving the INL98 and the upper bound of the 95% CI for deriving the UL. Based on the simulated individual values, the percentage of individuals reaching a serum 25OHD of 28 nmol/L (for INL98) and of 200 nmol/L (for UL) was calculated. Sensitivity analyses were performed assuming a non-skewed distribution, a CV of 0.40, as well as thresholds other than 28 and 200 nmol/L (i.e. 20, 25, 30, 35, 50 and 150, 180, 190, 210, 220, 250) and using as the mean of the inter-individual distribution, the 95%CI and 95%PI bounds in addition to the predicted study mean.

Results

Characteristics of the studies

A total of 31 studies of children aged two weeks to 3.9 years were included for the present modelling work (25,30–58), of which 26 were already identified in the original systematic review (16) and 5 from other guidelines or reviews (see **Figure 2**). The characteristics of the included studies are shown in **Table 2**. The mean age of the children within the studies was 6 months, with most of the studies (N=28) initiated in children below 6 months of age. The total duration of the trials ranged from 4 weeks to 24 months, with some trials providing intermediate measurements after a minimum 2 weeks since the start of the intervention. Total vitamin D intakes ranged from 0.6 to 57 µg/day (median of 11 µg/d; mean of 15 µg/d), including supplemental vitamin D ranging from 0 to 50 µg/d. The supplemental vitamin D forms used were vitamin D_3 in 20 studies, vitamin D_2 in 3 studies, both vitamers (i.e. vitamin D_2 compared to vitamin D_3) in 2 studies, and unspecified in 6 studies. Of the 31 RCTs, 29 used vitamin D supplements only and two used either breast milk from mothers who were supplemented or vitamin D supplements plus or minus vitamin D-fortified infant formula. The included studies were conducted in North America (N=11), Europe (N=9), Asia (N=8), and Australasia/Oceania (N=3), with latitudes ranging from 38°S to 61°N. No studies were identified from Africa or South America. The measures in the studies were taken across multiple seasons for 47% of the data points, in Winter for 8%, in Spring for 8%, in Summer for 6%, in Autumn for 4%, and season was not reported for 26% of the data points. In terms of skin pigmentation, 45% of the studies were conducted in mixed skin types, 13% in light skin types, while data on skin pigmentation was not reported in 42% (but were probably a majority of light or medium skin pigmentation). The method of serum 25OHD measurement were enzyme immunoassay (EIA)/Chemiluminescence (10 studies), competitive protein binding assays (9 studies), radioimmunoassays (6 studies), liquid chromatography tandem mass spectrometry (LC-MS/MS) (3 studies), high performance liquid chromatography (HPLC) (2

studies), and not reported in one study. Only 6 of the 31 included trials participated in an external quality assessment scheme for serum 25OHD measurement.

The detailed risk of bias assessments and strength of evidence assessed by GRADE are shown in **Supplementary Figure 1** and **Supplementary Tables 1 and 2**. The overall risk of bias was low in 6 studies, some concerns in 12 studies and high in 13 studies. The risk of bias was most often high due to deviations from the intended intervention, e.g. participants and/or personnel were aware of the intervention received (15 studies) or because of inappropriate analysis used to estimate the effect of assignment to intervention **(**15 studies). The overall strength of evidence was considered low, due to the risk of bias in the included studies, the paucity of standardized 25OHD measurements and studies in darker skin individuals, as well as the high heterogeneity between the studies, which covariates (such as latitude, season and skin pigmentation) could not explain significantly.

Unadjusted multi-level meta-regression modelling: the total vitamin D intake – serum 25OHD dose-response relationship

The best fit (i.e. the lowest AIC) was obtained with the cubic model. However, the cubic term was not significant. The second best fitting model was the quadratic model, which was selected for the further analyses, also because of its biological plausibility (the increase of 25OHD by vitamin D unit dose is larger at low intakes of vitamin D and lower at higher intake levels – see **Figure 3**). The log model showed the highest AIC. An overview of the models tested and their results are shown in **Supplementary Table 3**.

The possibility of fitting different models separately the overall age group of 0-3.9 years into infants and young children age was also explored. However, age was not significant when included as continuous variable in the model. In addition, when meta-regressions were conducted for different age categories individually (i.e. <6 months, 6-11.9 months, 0-11.9 months, and 0.5-3.9 years), model parameter estimates were very similar within the vitamin D

intake range of 10-45 µg/d. At the lowest and highest intakes, the age categories models diverged, due to the lack of data points (see **Supplementary Figure 2**).

The main quadratic model on 0-3.9 year old children was also run with data from 10 additional studies of children aged 4 to 9 years (59–68) to investigate whether adding supplementary evidence could improve the model at a potential expense of increasing the uncertainty in terms of reflecting the true relationship in the target population (0-3.9 years children). However, inclusion of these addition studies did not significantly change the shape of the model. The final model selected was the quadratic unadjusted model for children 0-3.9 years of age shown in **Figure 3**.

Inclusion of different covariates and their combinations (infant age, baseline 25OHD, region, country income category, 25OHD assay, season, skin pigmentation, and latitude) did not improve the model fit significantly or explained a significant part of the heterogeneity.

Inter-individual variability component: the full integrated model for INL98 and UL

The predicted percentage of young children reaching the serum 25OHD thresholds of 28 nmol/L and 200 nmol/L, associated with INL98 and UL respectively, at selected vitamin D intakes are shown in **Table 3** and **Figure 4**. The predicted percentage of individuals achieving the INL98-associated serum 25OHD threshold of 28 nmol/L ranged from 97.3% at 10 µg/d vitamin D intake to 99.1% at 60 µg/d. The predicted percentage of individuals exceeding the UL-associated serum 25OHD threshold of 200 nmol/L ranged from 0% at 10 µg/d vitamin D intake to 3.7% at 60 µg/d.

The findings of sensitivity analyses are shown in **Supplementary Tables 4-6**. The differences with the main analysis are limited except for the cases when using the 95%PI bounds as mean of the inter-individual distribution. However, this approach was considered over-conservative and not reliable for the setting vitamin D INL98 and UL and the model results overall considered robust.

Discussion

This paper presents the results from a novel multi-level and multivariable modelling of the response of serum 25OHD to total vitamin D intake in children aged below 4 years, including an inter-individual variability component. Our findings suggest that a vitamin D intake of 10 µg/day is required to maintain serum 25OHD concentrations in the vast majority (97.3%) of the young children over 28 nmol/L (i.e. threshold associated with minimised risk of rickets), corresponding to an INL98. From a safety perspective, the present analyses suggests that vitamin D intakes below 35 µg/day would keep serum 25OHD concentrations in almost all young children (98.6%) below 200 nmol/L, as the upper threshold associated with the UL for this age-group.

The vitamin D requirement estimates arising from this work differ partly from previous recommendations (see **Supplementary Table 7**), due to differences in the body of evidence used, the thresholds selected, the analyses conducted, and the type of recommendations derived. Compared to the 2004 WHO vitamin D recommendation (5 µg/day) for infants and young children (1), these new estimates, arising from the current modelling for the FAO-WHO update exercise, represent a more data-driven derivation of the vitamin D dietary requirement. While the serum 25OHD targets in the present analyses (of 28 nmol/L) and that of the 2004 recommendations (27 nmol/L) were extremely close, the former stem from a systematic review and individual participant data meta-analysis (15), whereas the latter was based on the prevailing view of the level necessary to ensure normal bone health as well as being the lower limit of the normal range (1). The present analyses used meta-regressive modelling to relate vitamin D intake to serum 25OHD, which also included an inter-individual variability component allowing for the estimation of the intake required to maintain serum 25OHD >28 nmol/L in 97.3% of young children. In contrast, the 2004 WHO recommendations relied on a more simplified approach that involved the estimation of the mean group dietary intake of

vitamin D required to maintain the plasma 25OHD levels above 27 nmol/L (1). In this method, the dietary intake of vitamin D for each population group was rounded to the nearest 50 IU (1.25 µg) and then doubled to cover the needs of all individuals within that group, irrespective of sunlight exposure. Notably, in the case of infants and young children the mean intakes were based on only a few studies overall. The 2004 WHO report on nutrient requirements did not establish a UL for vitamin D but noted that the adverse effects of high vitamin D intakes - hypercalciuria and hypercalcaemia - did not occur at the recommended intake levels proposed in the report (1).

The present INL98 vitamin D estimate cannot be directly compared with the international vitamin D reference values from IOM for North America (5) or EFSA for Europe (7). The modelling approach underpinning all three sets of vitamin D requirement estimates differed in various aspects, especially in relation to serum 25OHD thresholds (28 versus 40 and/or 50 nmol/L), use or non-use of covariates, and with respect to eligible RCT data - use or non-use of restrictions in relation to latitude (>40 or 49.5^{o}N), winter-time only RCTs, ethnicity of RCT participants, amongst other differences (5,7). The present INL98 vitamin D estimates for children aged 0-3.9 years stemmed from the multi-level and multivariable modelling, which included inter-individual variability simulations, whereas the estimates from IOM and EFSA's modelling were restricted to children aged 1 year and older; for infants both agencies set their vitamin D recommendations based on two vitamin D supplementation trials in breastfed babies (5,7). In addition, while the derivation of the UL for vitamin D was not based on meta-regression modelling in the case of the IOM reference values, it was in the case of EFSA's UL for infants up to 1 year of age. EFSA fitted a meta-regression dose-response model and adjusted for baseline 25OHD, integrating an inter-individual variability component, to predict the percentage of infants with serum 25OHD above 200 nmol/L at different vitamin D intakes, to establish an UL (27). The method used by EFSA to add this inter-individual variability

component in the model was taken and adapted for the present analysis, not only to derive a UL, but also an INL98.

The present work had a number of weaknesses. Firstly, many of the included studies had evidence of high bias and the certainty of the evidence was considered low. Evidence of high bias amongst the collection of RCTs used in vitamin D requirement derivation was also noted by EFSA in their exercise (7). Secondly, vitamin D intake from the general diet, which was added to vitamin D provided by the supplements or fortified foods to calculate the total vitamin D intake, had to be imputed from other sources for several studies. While this need to impute data on dietary intake is a limitation, it is one outweighed by the benefit for accounting for dietary supply of vitamin D from background diet to the estimate of total vitamin D intake. Thirdly, the analysis did not have estimates of vitamin D cutaneous synthesis and relied instead on indirect measures of potential UVB availability, such as latitude and season. However, even using these two proxies of cutaneous vitamin D synthesis did not provide major additional insight into the role of sunlight exposure when setting vitamin D intake requirements, similar to the experience of EFSA (7). While skin pigmentation is also an important factor that can affect cutaneous vitamin D synthesis, this was explored but could not be informatively included in the score calculations, since 42% of studies did not report (but were probably a majority of light or medium skin pigmentation), 45% were reported as mixed,13% reported as light skin type, and none reported dark skin type only. The majority of the studies were conducted in countries where the predominant racial group is white. This is an inherent limitation of the data rather than of the analysis. Nevertheless, this limitation should be a consideration as agencies make local context adjustments to these new estimates. In this regard, one cautious interpretation of the present vitamin D intake estimates is that they are most protective of those young children not synthesizing vitamin D in the skin. In addition, this analysis did not include premature and low birthweight infants, which can represent a significant portion of the infant

population, and which should be considered when interpreting our findings. Moreover, method-related differences in the measurements of serum 25OHD (69) were likely to have contributed additional variability to modelling of the vitamin D intake and serum 25OHD dose-response in the present work, as it has for other vitamin D recommendations from competent authorities (5–9). Standardization protocols exist to harmonize existing serum 25OHD data, but these are for observational-type studies (70), and for RCTs it would mean re-analysis of serum 25OHD samples using a certified LC-MS/MS method (71), which was beyond the scope of the present exercise. However, the data used to inform the inter-individual variability within the modelling was based on standardized 25OHD data. This data nonetheless were still a surrogate for empirical variability data from the 31 included RCTs which were not available. In the absence of availability of individual data from the RCTs, the inter-individual variability distribution shape and skewness was derived from one study and applied to the entire range of 25(OH)D predicted mean and corresponding vitamin intake. Although this approach represents a limitation since not based on real distributions observed in the studies, to our knowledge no better methods are available at the moment and frequently the issue of inter-individual variability is ignored. Another weakness is that the data was extracted by a single reviewer and not 2 independent reviewers, however the risk of errors was minimized by the thorough verification by a second reviewer. Lastly, while the literature search covered the period from inception to June 2020, it will have missed additional studies which would be likely illegible for inclusion in the modelling (72,73). This was outside the control of the present authors, as allocated resources within the exercise was such that the present work begun some after the original review (16) was completed. Furthermore, the collection of eligible RCTs in the present work is a major advancement over that collected in previous vitamin D intake requirement exercises.

The present work also has some important strengths which included the use of data coming from various steps within the overall risk assessment framework which either framed or facilitated the present modelling. This included, for example, evidence around the robustness of using 25OHD, the definition of serum 25OHD threshold for the minimization of nutritional rickets in young children (15), and key new exposure data from two systematic reviews on breast milk vitamin D content and breast milk intake volume (13,14). The vitamin D RCT data was also identified to a large extent from an independently commissioned systematic review and which was further refined in terms of use for the present modelling. The modelling used in the present work was comprehensive and goes beyond that of previous vitamin D requirement exercises, especially by including evidence from the entire globe and by its incorporation of inter-individual variability component.

This review also highlights a number of key research gaps which should be addressed going forward, more precisely the lack of published data from Africa and South America, the limited data available for children aged 1 to 3.9 years compared to up to 1 year of age, and further investigation of the role of ethnicity, sun exposure, as well as prematurity and low birthweight on dietary vitamin D intake estimates.

In conclusion, the present analysis provided new global estimates of vitamin D intake requirements (INL98 and UL) for children below 4 years of age. These new estimates can be used by countries across the globe once appropriate, local context adjustments (such as contribution to vitamin D status from sun exposure) are made to suit the intended population.

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Author contributions: KC and MRL screened the studies. MRL collected and extracted the data and KC verified them. LM and MRL conducted the analyses. KC, LM and MRL wrote the manuscript. All authors have read and approved the final manuscript

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Figure legends

Figure 1. Illustration of the steps used to model total vitamin D intake and serum 25OHD and to derive vitamin D requirements. Included RCTs provided aggregated data with total vitamin D intake and achieved study mean serum 25OHD (A). The relationship between vitamin D intake and serum 25OHD was modelled with a random effects multi-level meta-regression dose-response models (B). The impact of inclusion of potential covariates that could play the role of modifiers of this vitamin D intake-status relationship was tested with adjusted models. A target serum 25OHD threshold of 28 nmol/L was used as the basis of derivation of the INL98 for children aged 0-3 years, whereas an upper threshold of 200 nmol/L was used as the basis of derivation of the UL for children aged 0-3.9 years (C). Inter-individual variability of the response of serum 25OHD at different vitamin D intake levels were simulated (D). The modelling approach was used to estimate the vitamin D intake needed to maintain 98% of individuals over a stated serum 25OHD threshold concentration (INL98) and the vitamin D intake, which is judged to be unlikely to lead to serum 25OHD concentrations associated with adverse health (UL) (E).

Figure 2. Study selection flowchart

Figure 3. Relationship between total vitamin D intake [µg/d] and serum 25OHD [nmol/L] on 0 to 3.9 year old children fitted with unadjusted quadratic multi-level meta-regression. Black round dots represent the observed study arm means (N=186 data points). Blue line represents the mean response, the light blue fill represents the 95% confidence interval, and the light grey fill represents the 95% prediction interval.

Figure 4. Inter-individual variability distribution at vitamin D intake of 10 and 35 µg/day. Both inter-individual distributions were simulated with left-truncated normal, CV of 0.34, and 0.10 right-skewness. The inter-individual distribution at vitamin D intake of 10 µg/d, simulated using the mean predicted response from the unadjusted quadratic multi-level meta-regression (blue distribution), illustrates that nearly 98% of the individuals would achieve a serum 25OHD of 28 nmol/L, providing a basis for setting the INL98. The inter-individual distribution at vitamin D intake of 35 µg/d, simulated with the upper bound 95% CI of the predicted response from the unadjusted quadratic multi-level meta-regression (red distribution), illustrates that less than 2% of the individuals would achieve a serum 25OHD of 200 nmol/L or above, providing a basis for setting the UL. Dotted blue line represents the lower threshold of 28 nmol/L used to derive the INL98. Dotted red line represents the upper threshold of 200 nmol/L used to derive the UL. Black round dots represent the observed study arm means. Solid black line represents the predicted mean response, the dark grey fill represents the 95% confidence interval, and the light grey fill represents the 95% prediction interval.

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Tables

Table 1. Eligibility criteria for randomized controlled trials to contribute data for vitamin D intake requirement modelling

	Inclusion	Exclusion
Participants	Healthy children (included children with vitamin D deficiency) from 2 weeks of age up to 3 years (extended to 9 years to make sure sufficient data was available, as a sensitivity analysis)	Children with diseases (e.g. rickets) and conditions (very preterm and low birth weight)
Intervention	Daily vitamin D supplements or fortified foods, with a follow-up of minimum 2 weeks	Weekly, monthly, single dose vitamin D supplements or injections
Comparator	Low or zero vitamin D comparator	Invalid comparator (e.g. meat) or unable to isolate effect of vitamin D (e.g. with calcium in all groups)
Other	Maternal vitamin D supplementation ≤12.5 µg/day	Maternal vitamin D supplementation >12.5 µg/day

Table 2. Overview of included randomized controlled trials contributing data for vitamin D intake requirement modelling

First author date (Reference)	Country	Mean age (range) at baseline	Intervention description (dose) [a]	Follow-up	Adherence/ Compliance [b]	Vitamin D intake estimation	25OHD assay	Sun exposure	Skin pigmentation
Aglipay 2017 (30)	Canada	2.7 years (1-5 years)	Daily child vitamin D_3 supplementation (10 or 50 µg/d)	4 months	100% and 98%	Estimated from study in similar population (61)	Protein-binding assay	Low (43°N; Trial during winter; 35-60 min unstructured free play outdoors per week at baseline)	Multiple (Fitzpatrick skin type 13% I, 31% II, 33% III, 11% IV, 6% V, 4% VI)
Ala-Houhala 1985 (31)	Finland	Birth	Daily child vitamin D supplementation (10 or 25 µg/d)	8, 20 weeks	NR	Estimated from breast milk concentration (74) and intake (14)	Competitive protein binding assay	Variable (61°N; groups in winter and summer)	Light (largely fair skin color)
Ala-Houhala 1986 (32)	Finland	Birth	Daily child vitamin D_3 supplementation (0 or 10 µg/d) for 15 weeks	8, 15 weeks	NR	Estimated from breast milk concentration (74) and intake (14)	Competitive protein binding assay	Low (61°N; recruited in January)	Light (largely fair skin color)
Alonso 2011 (33)	Spain	1 month	Daily child vitamin D_3 supplement	3, 6, 12 months	Excluded non-compliant	Estimated from study in similar	EIA/Chemiluminescence	Variable (43°N; recruited over 1 year;	Light/Medium (excluded dark skin

			ation (0 or 10 µg/d)			population (75)	excluded children with sunlight exclusion)	pigmentation)
Atas 2013 (34)	Turkey	15 days	Daily child vitamin D_3 supplementation (5 or 10 µg/d)	4 months	Excluded infants lost to follow-up and improper vitamin D supplementation	Estimated from breast milk concentration (13) and intake (14)	HPLC	Variable (40.6°N; recruited over 1 year)	Probably medium (Middle East)
Chan 1982 (35)	USA	2 weeks	Human milk with daily maternal supplementation (vitamin D 10 µg/d and calcium 250 mg/d) or human milk with daily child vitamin D supplementation (10 µg/d) or vitamin D fortified	2, 4, 6 months	NR	Reported in study and estimated from breast milk concentration (13) and intake (14)	Competitive protein binding assay	Probably low (40.8°N; no seasonal variation was found in the study)	Light (Caucasian)

			formula (vitamin D 10 µg /L, calcium 0.51 mg/dL and phosphorus 0.39 mg/dL)					
Chandy 2016 (36)	India	2-4 days	Daily child vitamin D_3 supplementation (0 or 10 µg/d)	3.5 months	94%	Estimated from breast milk concentration (13) and intake (14)	RIA kits	Probably significant (26°N; mothers were instructed to give baby massage under the sun 15 min per day)	Probably medium (India)
Enlund-Cerullo 2019 (37)	Finland	2 weeks	Daily child vitamin D_3 supplementation (10 or 30 µg/d)	12, 24 months	89% and 87%	Estimated from study in similar population (76)	EIA/Chemiluminescence [c]	Probably variable (60.1°N; recruited over several times of the year)	Probably light (mothers of Northern European origin)
Gallo 2013a (38)	Canada	1 month	Daily child vitamin D_2 or D_3 supplement	3 months	89%	Estimated from study in similar	LC-MS/MS [c, d]	Probably variable (45.5°N; recruited	Multiple (67% self-identified as White, Skin

			ation (10 µg/d)			population (39)		over more than 1 year; 58% infants born during vitamin D-synthesizing period April-October)	color based on ITA: 10% very fair, 46% fair, 35% medium, 6% olive, 4% dark)
Gallo 2013b (39)	Canada	1 month	Daily child vitamin D_3 supplementation (10, 20, 30 or 40 µg/d) for 12 months	1, 2, 5, 8, 11 months	84-93%	Reported in study	LC-MS/MS [c, d]	Probably variable (45.5°N; recruited over more than 1 year; 60% infants born during vitamin D-synthesizing period April-October; Sun exposure did not differ between groups but infant sun index increased from 7 at 1	Multiple (84% White)

							mo to 71 at 9 mo old)	
Gordon 2008 (25)	USA	10 months (8-24 months)	Daily child vitamin D_2 or D_3 supplementation (vitamin D_2 50 µg/d or vitamin D_3 50 µg/d, both groups received calcium 50 mg/kg/d)	6 weeks	NR	Estimated from study in similar population (77)	EIA/Chemiluminescence	Probably variable (42°N; recruited over the year)	Multiple (skin pigmentation 1 (heaviest) 62%, 2 27%, 3 4%, 4 (lightest) 8%)
Grant 2014 (40)	New Zealand	Birth	Daily child vitamin D_3 supplementation (placebo 0 µg/d) for 6 months	2, 4, 6 months	78-90%	Estimated from study in similar population (39)	LC-MS/MS[c]	Probably variable (36°S; recruited at all times of the year; average time spent outdoors 0.21 h/d at 2 mo, 0.25 h/d at 4 mo, and 0.40 h/d at 6 mo)	Multiple (Mother 38% European, 24% Maori, 46% Pacific, 25% Other)
Greer 1982 (41)	USA	3 weeks	Daily child vitamin D supplement	3, 9, 23 weeks	80%	Estimated from breast milk	Competitive protein	Probably variable (43°N;	Light/Medium (94% Caucasian,

			ation (0 or 10 µg/d)			concentration (13) and intake (14)	binding assay	unclear season; sunshine exposure 35 min/d)	6% Asian-Indian)
Hollis 2015 (42)	USA	5 weeks (4-6 weeks)	Daily child and maternal vitamin D_3 supplementation (10/10 µg/d)	3, 6 months	NR	Estimated from breast milk concentration (13) and intake (14)	RIA kits	Probably variable (38°N; recruited over different times of the year)	Multiple (59% White, 22% Hispanic, 19% Black/African American
Holst-Gemeiner 1978 (43)	Austria	1 week (2-10 days)	Daily child vitamin D_3 supplementation (30 µg/d)	2, 4-6 weeks	NR	Estimated from breast milk concentration (13) and intake (14)	RIA	Probably low (48°N; newborns)	Probably light (Western Europe)
Huynh 2017 (44)	Australia	Birth	Daily child vitamin D_3 supplementation (10 µg/d)	3-4 months	69%	Estimated from breast milk concentration (13) and intake (14)	EIA/Chemiluminescence	Probably low (38°S; considered minimal by authors)	Multiple (Maternal skin pigmentation 50% light-olive, 50% dark)
Kunz 1982 (45)	Germany	Birth	Daily child vitamin D_3 supplementation (12.5 or 25 µg/d)	6 weeks	NR	Estimated from breast milk concentration (13) and intake (14)	Protein-binding assay	Probably low (48°N; season not reported; newborns)	Probably light (Western Europe)

Madar 2009 (46)	Norway	6 weeks	Daily child vitamin D_2 supplementation (0/usual care or 10 µg/d)	7 weeks	91%	Estimated from breast milk concentration (13) and intake (14)	HPLC-APCI-MS [c]	Probably low (60°N; all seasons, no differences in 25OHD found between seasons)	Medium/Dark (Pakistani, Turkish or Somali)
Pehlivan 2003 (47)	Turkey	2 weeks	Daily child vitamin D supplementation (10 or 20 µg/d)	4 months	NR	Estimated from Global Dietary Database	EIA/Chemiluminescence	Probably low (40.8°N; according to authors sunlight exposure is low due to dressing habits, low vitamin D dietary intake, and air pollution; time of year not mentioned, except for control group; maternal vitamin D	Probably medium (Middle East)

							intake and dressing habits were correlated with 25OHD, correlations for infant 25OHD were not reported)	
Pittard 1991 (48)	USA	Birth	Daily child vitamin D supplementation (10 or 20 µg/d)	2, 4, 6, 8, 10, 14, 16 weeks	NR	Reported in study	Competitive protein binding assay	Probably variable (32.8°N; time of year not mentioned)	Multiple (20% White, 80% Black)
Ponnapakkam 2010 (49)	USA	Birth	Daily child vitamin D_3 supplementation (0 or 5 µg/d from birth or starting at 2 months)	2, 4, 6 months	82%	Estimated from study in similar population (77)	EIA/Chemiluminescence	Variable (30°N; across several times of the year; differences in skin color and clothing was equally distributed between groups at	Multiple (dark skin color was distributed evenly between groups at randomization)

									randomization)
Rueter 2019 (50)	Australia	<28 days	Daily child vitamin D_3 supplementation (0 or 10 µg/d)	3, 6 months	NR	Estimated from study in similar population (39)	EIA/Chemiluminescence	Variable (32°S; recruitement across multiple seasons, no differences between seasons found; UV light exposure was measured in 42% of infants, was 1204 J/m2 in vitamin D group and 815 J/m2 in control group, was not correlated with 25OHD or season of birth)	Not reported

Shakiba 2010 (51)	Iran	Birth	Daily child vitamin D_3 supplementation (5 or 10 µg/d)	6 months	NR	Estimated from Global Dietary Database	EIA/Chemiluminescence	Probably variable (32°N; January-September)	Probably medium (Middle East)
Siafarikas 2011 (52)	Germany	4-5 days	Daily child vitamin D_3 supplementation (6.25 or 12.5 µg/d)	6 weeks	NR	Estimated from breast milk concentration (13) and intake (14)	RIA kits [c]	Low (52.5°N; recruited during summer and winter equally; absolute UVB exposure measured 2.5-20 J/m2)	Light (included only photo-types I and II according to Fitzpatrick and Bolognia)
Singh 2018 (53)	India	Birth	Daily child vitamin D_3 supplementation (0 or 10 µg/d)	6 months	NR	Estimated from breast milk concentration (13) and intake (14)	EIA/Chemiluminescence	Probably variable (29°N; January-September)	Probably medium (Southeast Asia)
Specker 1992 (54)	China	Birth	Daily child vitamin D supplementation (2.5, 5 or 10 µg/d)	6 months	96-131%	Estimated from Global Dietary Database	EIA/Chemiluminescence	Variable (22, 30, 40, 47°N; enrolled during fall and spring)	Probably medium (North and South China)
Vervel 1997	France	1 month	Daily child vitamin D_2	1.5-2, 2.5-4 months	NR	Reported in study and	Competitive protein	Probably variable	Not reported

(Study 1) (55)			supplementation (25 µg/d) with vitamin D fortified or non-fortified formula			combined with estimates from breast milk concentration (13) and intake (14)	binding assay	(49°N; measures at different time of year)
Vervel 1997 (Study 2) (55)	France	Birth	Daily child vitamin D_2 supplementation (12.5 or 25 µg/d) from mothers supplemented during pregnancy (0 or 12.5 µg/d)	3 months	NR	Reported in study and combined with estimates from breast milk concentration (13) and intake (14)	Competitive protein binding assay	Probably variable (49°N; recruited April-July)	Not reported
Wagner 2006 (56)	USA	1 month	Daily child and maternal vitamin D_3 supplementation (7.5/10 µg/d)	4, 7 months	≥61% and ≥80%	Estimated from breast milk concentration (13) and intake (14)	RIA	Probably low (33°N; Mothers were instructed to avoid direct sunlight exposure of their infants during the first 6 mo)	Multiple (Maternal ethnicity 11% African American, 74% White, 15% Hispanic)

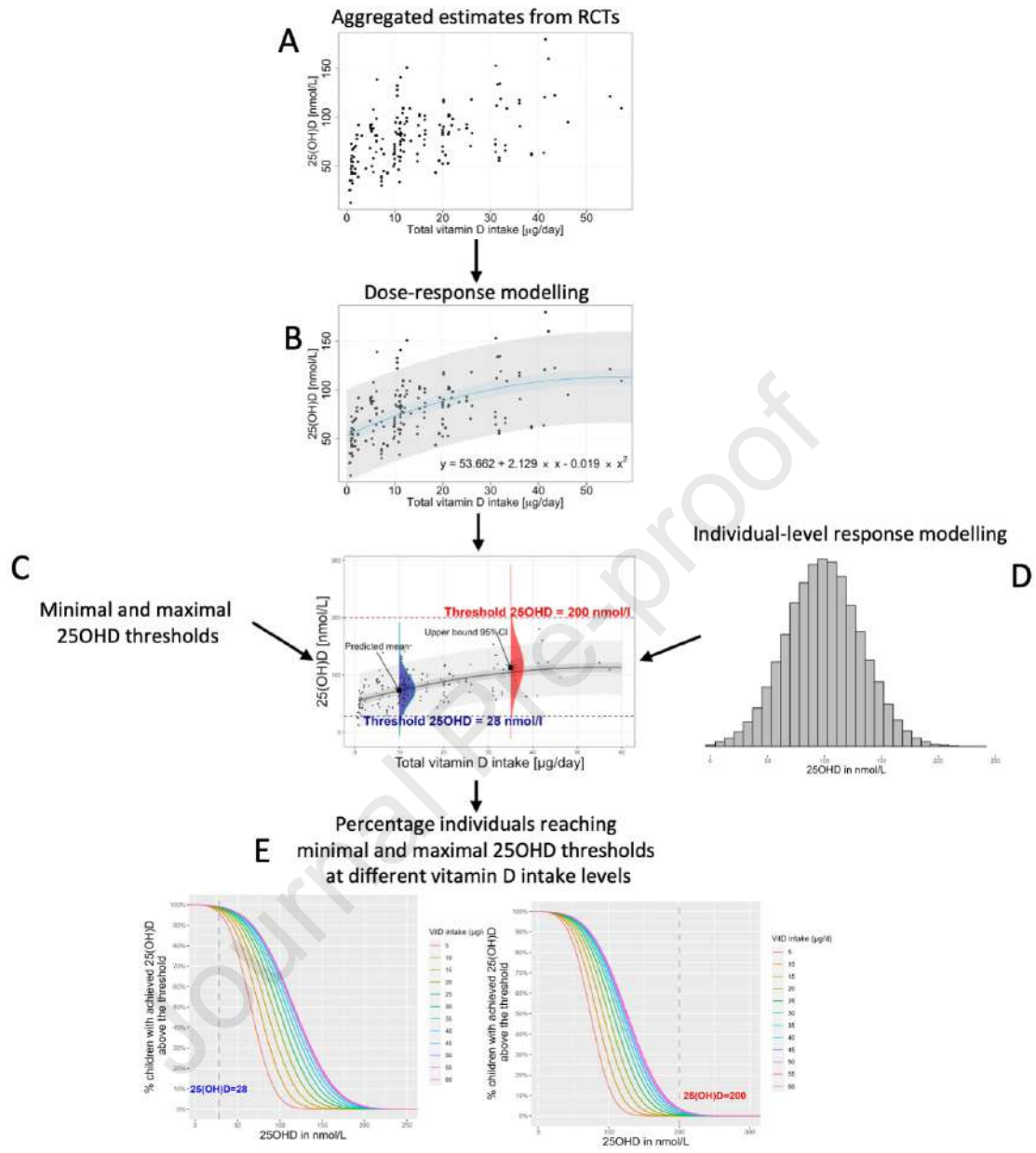
Zhou 2018 (57)	China	7.8 months (3-12 months)	Daily child vitamin D_3 supplementation (10 or 30 µg/d)	2, 4 months	Excluded non-compliant	Estimated from Global Dietary Database	Not reported	Variable (29°N; recruited over multiple seasons)	Probably medium (China)
Ziegler 2014 (58)	USA	24-32 days	Daily child vitamin D_3 supplementation (5, 10, 15 or 20 µg/d)	1, 3, 4.5, 6.5, 8, 11 months	103.40%	Estimated from breast milk concentration (13) and intake (14) and food intake from study in similar population (77)	RIA kits	Low (41°N; main assessment during winter, minimal sun exposure)	Multiple (90% White, 4% Hispanic, 3% African American, 2% Native American, 1% Asian)

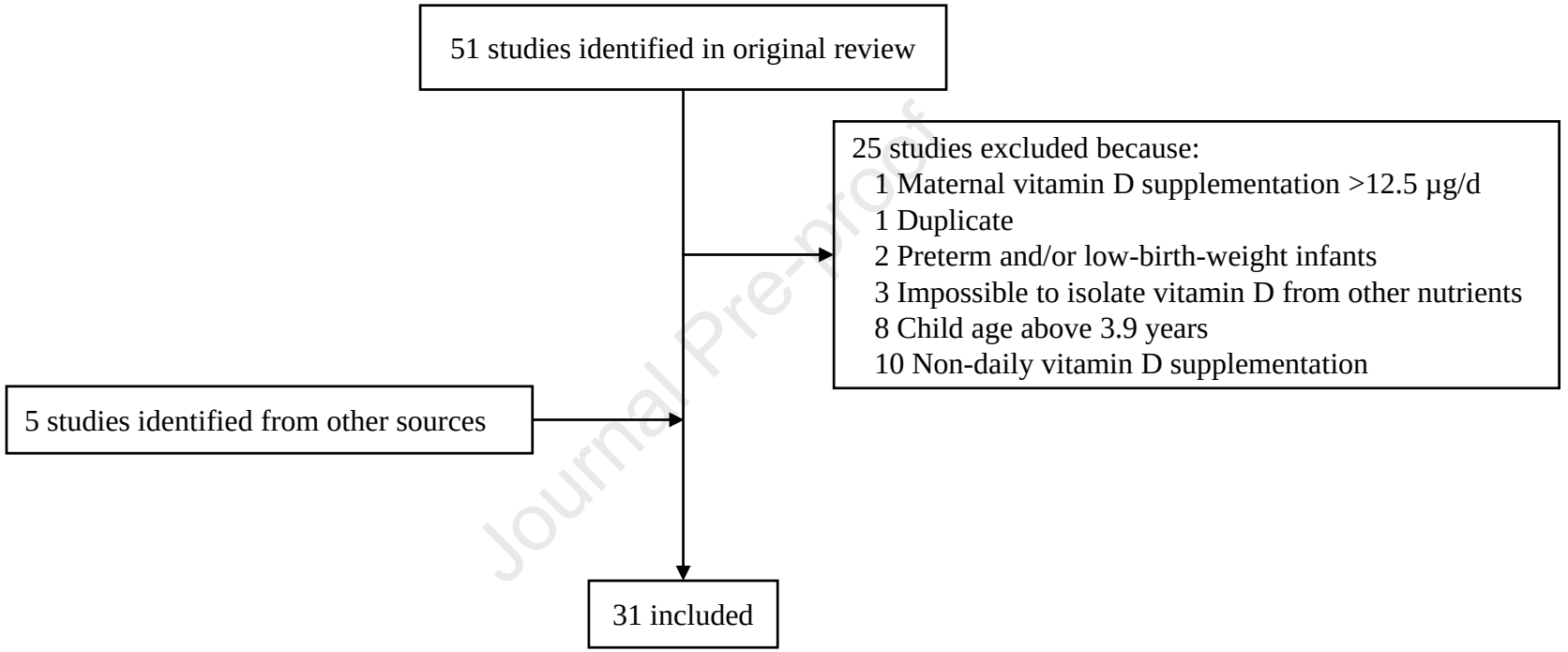
Notes: [a] The study arms which did not correspond to the inclusion criteria were excluded from the data analyses i.e. weekly, monthly, single dose vitamin D supplementation, impossible to isolate effect of vitamin D or maternal vitamin D supplementation <12.5 µg/d). [b] Expressed as a percentage of dose taken, unless stated otherwise. [c] Participated in an external quality assessment scheme for serum 25OHD measurement. [d] Also measured serum 25OHD with immunoassay. Abbreviations: 25OHD: 25-hydroxy-vitamin D; EIA: Enzyme immunoassay; HPLC: High-performance liquid chromatography; LC-MS/MS: Liquid chromatography with tandem mass spectrometry; NR: not reported; RIA: Radioimmunoassay.

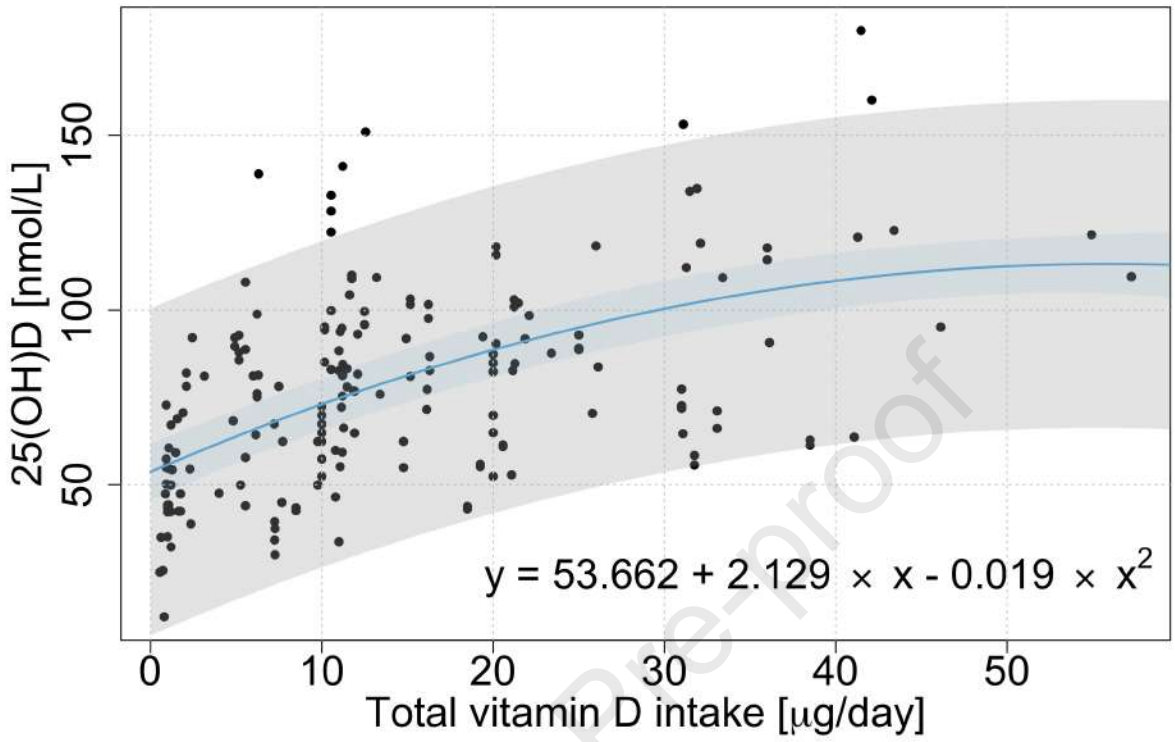
Table 3. Predicted percentage of individuals [%] reaching the serum 25OHD thresholds 28 and 200 nmol/L respectively (used to derive INL98 and UL respectively). Modelling with left-truncated normal distribution, right-skewed (0.10), CV=0.34 and predicted mean response and predicted upper bound of 95%CI mean response as the mean value of the inter-individual distribution respectively for INL98 and UL.

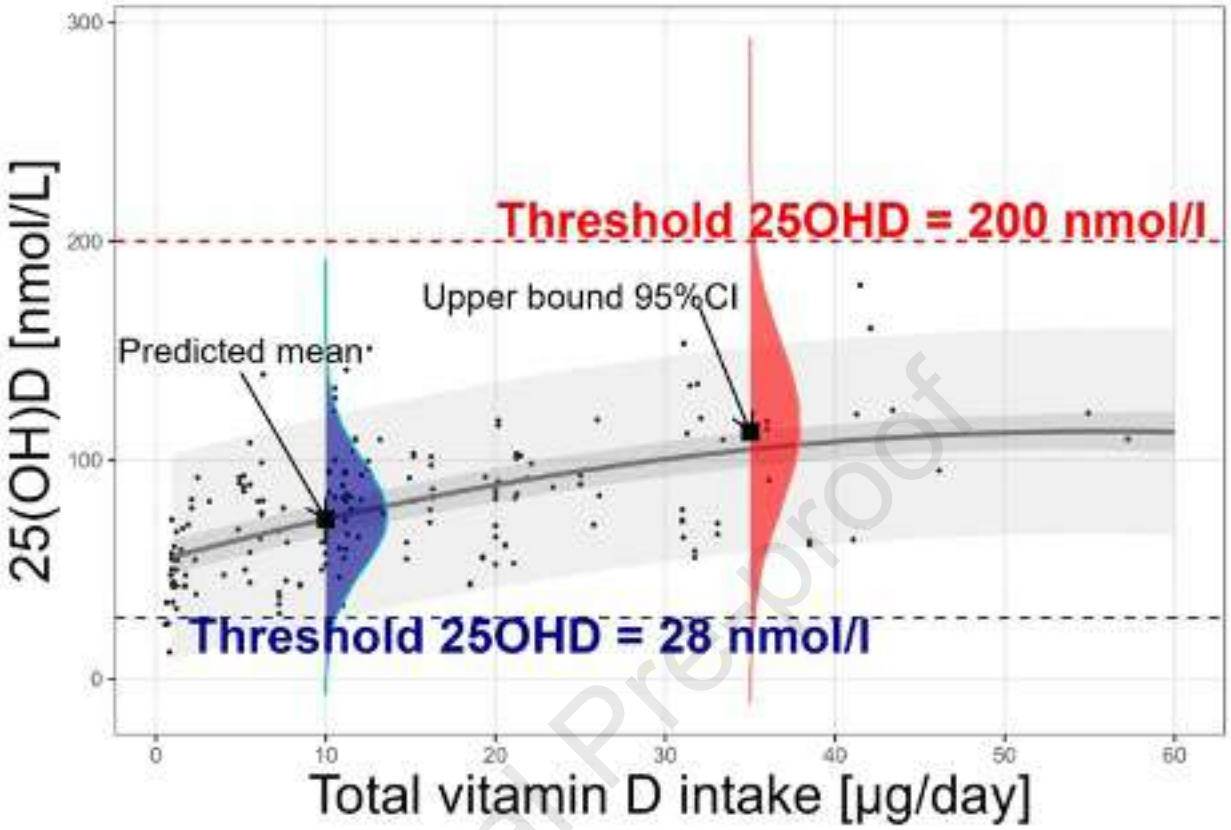
Vitamin D intake [µg/d]	Percentage individuals reaching serum 25OHD threshold of 28 nmol/L (used to set the INL98)	Percentage individuals reaching serum 25OHD threshold of 200 nmol/L (used to set the UL)
10	97.30	0.00
15	97.88	0.02
20	98.35	0.09
25	98.57	0.35
30	98.71	0.79
35	98.84	1.41
40	98.98	1.96
45	98.96	2.61
50	99.08	3.19
55	99.05	3.43
60	99.07	3.65

Abbreviations: CV: Coefficient of variation; INL98: : daily intake reference value that is estimated to meet the nutrient requirement of 97.5% of the apparently healthy individuals in a specific life stage and sex group; LBCI: lower bound 95% confidence interval; UBCI: upper bound 95% confidence interval; UL: upper limit; 25OHD: 25-hydroxyvitamin D.









Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Magali Rios-Leyvraz reports financial support was provided by the World Health Organization to conduct this work as an independent consultant.

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Laura Martino was an independent consultant on statistical issues with no financial support from the World Health Organization. The consultancy was performed on personal capacity.

Kevin D. Cashman was part of the FAO/WHO expert group. The individuals in the FAO/WHO expert group were required to declare a lack of conflict of interests.