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Estimation of the dietary requirement for vitamin D in white children aged 4–8 y: a randomized, controlled, dose-response trial[1,2]

Charlotte Mortensen,[3,4,9] Camilla T Damsgaard,[3,9] Hanne Hauger,[3] Christian Ritz,[3] Susan A Lanham-New,[5] Taryn J Smith,[5] Áine Hennessy,[6] Kirsten Dowling,[6] Kevin D Cashman,[6,7] Mairead Kiely,[6,8] and Christian Mølgaard[3]*

[3]Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark; [4]Department of Nutrition and Midwifery, Faculty of Health and Technology, Metropolitan University College, Copenhagen, Denmark; [5]Department of Nutritional Sciences, Faculty of Health and Medical Sciences, University of Surrey, Surrey, United Kingdom; [6]Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences, [7]Department of Medicine, and [8]Irish Centre for Fetal and Neonatal Translational Research, University College Cork, Cork, Ireland

ABSTRACT

Background: Children in northern latitudes are at high risk of vitamin D deficiency during winter because of negligible dermal vitamin D_3 production. However, to our knowledge, the dietary requirement for maintaining the nutritional adequacy of vitamin D in young children has not been investigated.

Objective: We aimed to establish the distribution of vitamin D intakes required to maintain winter serum 25-hydroxyvitamin D [25(OH)D] concentrations above the proposed cutoffs (25, 30, 40, and 50 nmol/L) in white Danish children aged 4–8 y living at 55°N.

Design: In a double-blind, randomized, controlled trial 119 children (mean age: 6.7 y) were assigned to 0 (placebo), 10, or 20 μg vitamin D_3/d supplementation for 20 wk. We measured anthropometry, dietary vitamin D, and serum 25(OH)D with liquid chromatography–tandem mass spectrometry at baseline and endpoint.

Results: The mean ± SD baseline serum 25(OH)D was 56.7 ± 12.3 nmol/L (range: 28.7–101.4 nmol/L). Serum 25(OH)D increased by a mean ± SE of 4.9 ± 1.3 and 17.7 ± 1.8 nmol/L in the groups receiving 10 and 20 μg vitamin D_3/d, respectively, and decreased by 24.1 ± 1.2 nmol/L in the placebo group ($P < 0.001$). A nonlinear model of serum 25(OH)D as a function of total vitamin D intake (diet and supplements) was fit to the data. The estimated vitamin D intakes required to maintain winter serum 25(OH)D >30 (avoiding deficiency) and >50 nmol/L (ensuring adequacy) in 97.5% of participants were 8.3 and 19.5 μg/d, respectively, and 4.4 μg/d was required to maintain serum 25(OH)D >40 nmol/L in 50% of participants.

Conclusions: Vitamin D intakes between 8 and 20 μg/d are required by white 4- to 8-y-olds during winter in northern latitudes to maintain serum 25(OH)D >30–50 nmol/L depending on chosen serum 25(OH)D threshold. This trial was registered at clinicaltrials.gov as NCT02145195. *Am J Clin Nutr* 2016;104:1310–7.

Keywords: children, dose-response, ODIN, randomized controlled trial, recommendations, requirement, vitamin D

INTRODUCTION

Adequate vitamin D status is important in supporting bone growth and development during childhood (1–3). The Institute of Medicine (IOM)[10] in the United States (4) and the Scientific Advisory Committee on Nutrition in the United Kingdom (5) have suggested that a serum 25-hydroxyvitamin D [25(OH)D] concentration <30 and <25 nmol/L, respectively, indicates an increased risk of vitamin D deficiency and its associated risk of bone disease. Some European agencies (6, 7) have proposed that a serum 25(OH)D concentration of 50 nmol/L is required to cover the needs of most children in relation to bone health outcomes. The IOM has proposed that a serum 25(OH)D concentration of 40 nmol/L covers the mean requirement and that a concentration of 50 nmol/L covers the needs of 97.5% of the population, thus exceeding the requirements of most individuals in the life-stage group (4). Recent data from the first internationally comparable prevalence estimates of vitamin D deficiency in Europe suggest that 13% of European children and adults have serum 25(OH)D <30 nmol/L and that 40% have serum 25(OH)D <50 nmol/L (8). These estimates are much higher during an extended winter period (October to March) (8), when dermal synthesis is negligible in northern latitudes (9). Although sufficient dietary intake of vitamin D can offset the lack of dermal synthesis, median intakes in children in Northern European countries such as Denmark, the United Kingdom, and Ireland have been shown to be low at ~2 μg/d (10–12), in line with more global data (13).

The Dietary Reference Intakes (DRIs) set by authoritative agencies are based on serum 25(OH)D thresholds ranging from 30

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[2] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

[9] These authors contributed equally to this work.

*To whom correspondence should be addressed. E-mail: cmo@nexs.ku.dk.

[10] Abbreviations used: DRI, Dietary Reference Intake; EAR, Estimated Average Requirement; FFQ, food-frequency questionnaire; IOM, Institute of Medicine; ITT, intention to treat; LC-MS/MS, liquid chromatography–tandem mass spectrometry; ODIN, Food-Based Solutions for Optimal Vitamin D Nutrition and Health through the Life Cycle; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

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to 50 nmol/L (4–8). However, these agencies had few data available on which to base vitamin D status-intake dose-response models to support requirement estimates. Of the 3 randomized controlled trials in children and teenagers used in the IOM exercise (4), 1 trial was conducted in only 20 participants (mean age: 9.8 y) and for only 4 wk (14) and another was conducted in 1988 (mean age: 9 y) (15). The largest of the studies was conducted in 2006 in 226 adolescent girls (16). This is a small number of trials on which to base DRIs in children and the assumption of no age dependency in the dose-response relation, and the IOM and others have indicated that further research is warranted to report DRIs with confidence (4, 5). Data from controlled trials in younger children are lacking, and to our knowledge the actual dose-response relation between vitamin D intake and serum 25(OH)D has not been investigated in children <8 y (17).

Thus, the primary objective of this randomized, controlled, double-blind trial (NCT02145195) was to establish the distribution of dietary requirements for maintaining serum 25(OH)D concentrations ranging from ≥25 to ≥50 nmol/L in white Danish children aged 4–8 y during a 5-mo winter period. Identifying the intake values that will maintain serum 25(OH)D concentrations above chosen cutoffs during winter has been the approach by most agencies that establish dietary recommendations (4–6).

METHODS

Study design

The ODIN (Food-Based Solutions for Optimal Vitamin D Nutrition and Health through the Life Cycle) Junior study was part of the pan-European collaborative ODIN study. ODIN Junior was a randomized, double-blind, placebo-controlled dose-response trial. The design closely followed the design previously reported by Cashman et al. in adults (18, 19). A total of 130 Danish children aged 4–8 y living at 55°N were randomly assigned to receive 0 (placebo), 10, or 20 μg vitamin D_3/d for 20 wk. Randomization was performed in blocks of 12 children to make sure that they were evenly distributed in the 3 intervention groups throughout each 5-wk examination period. Measurements and blood sampling took place at baseline (29 September to 31 October 2014) and endpoint (11 February to 18 March 2015) after a median of 20 wk (range: 17.3–21.0) of intervention at University of Copenhagen, Denmark. The study protocol was approved by the Committees on Biomedical Research Ethics for the Capital Region of Denmark and conducted in accordance with the Declaration of Helsinki (20).

Subjects

Participants were recruited with the use of data from the Danish National Central Offices of Civil Registrations. Names and addresses of 15,000 children aged 4.0–8.8 y at baseline were identified, and from July to September 2014 a total of 3650 invitation letters were sent out to families in Copenhagen and Frederiksberg (55°N) (**Figure 1**). Some additional recruitment took place by personal networks. After screening for eligibility, 164 families were invited for informational meetings at which the study and its procedures were explained to children and their parents. A total of 130 children were included (7 pairs of which were siblings), and informed written consent was obtained from all custody holders.

Eligible subjects were white Danish or European children aged 4–8 y who were not planning a winter vacation south of latitude 51°N, corresponding to Surrey, United Kingdom, where a similarly designed ODIN trial was simultaneously conducted in adolescents aged 14–18 y. The exclusion criteria were diseases or intake of medicine known to affect vitamin D or calcium metabolism, the use of vitamin D-containing supplements ≥4 d/wk in the last 8 wk before intervention start and any use of vitamin D-containing supplements in the 4 wk before intervention start, concomitant participation in other studies with dietary supplements or blood sampling, and baseline serum calcium concentrations >2.7 mmol/L (hypercalcemia) (21), as assessed shortly after the baseline examination.

Intervention

Tablets, compliance, and blinding

Parents were supplied with 1 bottle of tablets (containing 170 tablets), a 7-d tablet dispenser, and tablet registration sheets to be filled out every week and were instructed to give the child 1 tablet/d during the intervention, preferably during breakfast. Minisun tablets were provided by OY Verman Ab and were chewable and slightly sweetened with xylitol and sorbitol. Placebo and vitamin D tablets were identical in taste and appearance. The tablets were packaged and coded in identical neutral bottles. The tablets were analyzed with the use of liquid chromatography–tandem mass spectrometry (LC-MS/MS) after the intervention by an independent laboratory (National Food Institute, Søborg, Denmark). The analysis showed that the placebo and 10- and 20-μg tablets contained <0.2, 10.9, and 22.4 μg vitamin D_3, respectively (SD: 2.8%; n = 5 tablets from each dose analyzed). Registration sheets and tablets that were not consumed were collected at endpoint, and compliance (%) was evaluated by counting the remaining tablets and calculated as the number of tablets consumed/number of intervention days × 100. Parents were asked about travel outside Denmark at midpoint after 9.7 wk (range: 8–12) of intervention by telephone and at the endpoint examination visit. Blinding of investigators and parents was checked by asking them to guess the child's intervention group at endpoint, and investigators were unblinded after the data were analyzed.

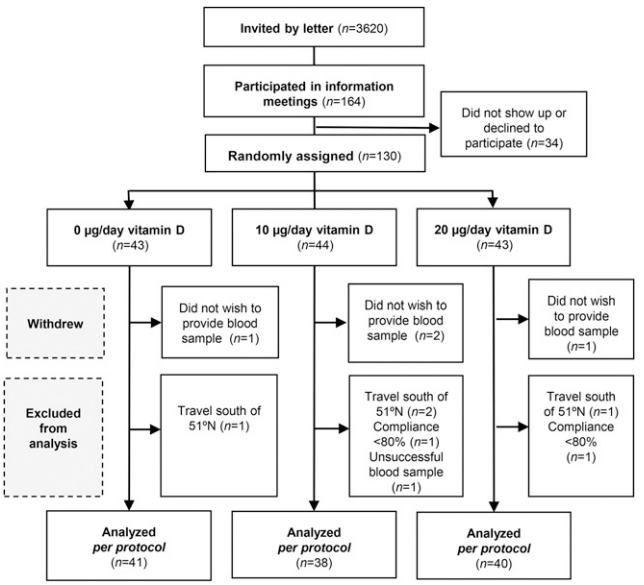

FIGURE 1 Flow diagram of study participants.

Randomization

A computer-generated list of consecutive study identification numbers, each linked to a tablet dose, was generated by a staff member not involved in the study or in the data analysis with the use of the statistical environment R (22). Tablet bottles were labeled with these continuous numbers to ensure that neither investigators nor participants knew who were in the same intervention group. Participants were assigned an identification number in the order they appeared at the baseline examination visit. Siblings were randomly assigned individually, and the parents were instructed to keep each child's tablets strictly separate within the household.

Measurements

Background information

At baseline, parents completed a questionnaire on parental education, and pubertal stage was evaluated in girls aged 7–8 y by a parent-administered questionnaire with drawings of breast development (Tanner stages I–V) (23). All boys and girls aged <7 y were assumed not to have reached puberty based on nationally representative data (24, 25).

Adverse events

At midpoint and at the endpoint examination visit, parents were asked whether their child's health had changed or whether there had been any potential adverse events related to the supplementation. All symptoms (former as well as ongoing) described by the parents were recorded. The responsible physician assessed recorded symptoms for severity and relation to supplementation. Unresolved symptoms reported at midpoint were specifically followed-up at endpoint.

Dietary intake of vitamin D and calcium

Dietary vitamin D and calcium intakes were estimated with the use of a food-frequency questionnaire (FFQ) that has previously been validated for these nutrients (26). The FFQ was administered by nutrition researchers and contained 14 questions covering 8 food items (milk, yogurt, cheese, cereals, bread, egg, meat, and fish). The chosen food items contribute to 95% of the vitamin D intake and 75% of the calcium intake in Danish children aged 4–8 y according to the 2010 national Danish dietary survey (27). The Danish Food Composition database (28, 29) was used to assign vitamin D composition values to the FFQ items and was supplemented by United Kingdom food composition tables (30) when a suitable Danish value was not available. Total vitamin D intake for each individual was calculated as the sum of dietary vitamin D intake as assessed by FFQ at endpoint and the supplemental vitamin D dose based on the analyzed content of vitamin D_3 in the tablets.

Anthropometry

Anthropometric measurements were performed while the children were wearing light clothing and after they had emptied their bladder. Height was determined to the nearest 0.1 cm with the use of a 235 Heightronic digital stadiometer (QuickMedical) while the child was standing barefoot with the head in the Frankfurt horizontal plane and calculated as the mean of 3 consecutive measurements. Weight was determined with a Tanita BWB-800 S digital scale to the nearest 0.1 kg. Waist circumference was determined to the nearest 0.1 cm at the level of the umbilicus, and the mean of 3 consecutive measurements was calculated. Sex- and age-adjusted *z*-scores for BMI (in kg/m^2) were calculated with the use of WHO AnthroPlus software version 1.0.4 (31).

Blood sampling

A 25-mL venous blood sample was drawn from the forearm of the child after a fast of ≥2 h. The content and timing of the breakfast meal before blood sampling at baseline was recorded, and the parents were instructed to serve the same meal to the child before the endpoint examination. Samples were centrifuged at 2300 × *g* for 10 min at 4°C, and the serum and plasma were stored at −80°C until analysis.

Analysis of serum 25(OH)D, parathyroid hormone, and calcium

Serum $25(OH)D_2$ and $25(OH)D_3$ were analyzed with the use of LC-MS/MS at the Cork Centre for Vitamin D and Nutrition Research. This LC-MS/MS method is traceable to the reference measurement procedures previously described (32). The quality and accuracy of serum 25(OH)D analysis is monitored on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme, and the Cork Centre's LC-MS/MS method is certified by the CDC's Vitamin D Standardization Certification Program. Total 25(OH)D was calculated as the sum of serum $25(OH)D_2$ and $25(OH)D_3$. The intra- and interassay CVs for the analysis were <5% and <6%, respectively.

Plasma parathyroid hormone (PTH) and serum calcium were analyzed, and PTH was measured in 1 batch on an Immulite 1000 (Siemens Medical Solutions Diagnostics). In total, 4 and 1 samples were below the detection limit of 0.316 pmol/L at baseline and endpoint, respectively, and defined as 0.158 pmol/L. The intra- and interassay CVs for the analysis were 3.8% and 1.3%, respectively. Serum calcium was analyzed shortly after the baseline examination and after the endpoint visit on a Pentra 400 (Horiba) and was corrected for albumin. Intra- and interassay CVs were 3.0% and 4.0%, respectively. Hypercalcemia was defined as uncorrected serum calcium concentration >2.7 mmol/L as suggested by Lietman et al. (21).

Serum 25(OH)D cutoffs

A serum 25(OH)D concentration <30 nmol/L was used to indicate vitamin D deficiency per the IOM (4). We also applied the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition cutoff of 25 nmol/L to indicate severe deficiency (33). Moreover, we used the IOM's suggested concentration of 40 nmol/L as that relating to the Estimated Average Requirement (EAR) (i.e., the nutrient intake for which 50% of a group meets the requirement) for vitamin D at 10 μg/d (4). In addition, we used the IOM's suggested concentration of 50 nmol/L as that relating to the Recommended Dietary Allowance of 15 μg/d, which covers the needs of 97.5% of the population, thus exceeding the requirements of most individuals in the life-stage group (4).

Power calculation

The power calculation was based on the expected slope of the relation between total vitamin D intakes and serum 25(OH)D in the study population. Because of the relative paucity of data on

the relation between habitual vitamin D intake and serum 25(OH)D concentrations in this age group, power calculations were performed under relatively pessimistic assumptions concerning the magnitude of any relation and the residual variation in serum 25(OH)D concentration after the effect of background dietary intake has been removed. Specifically, a value of 1.0 was assumed to represent the minimum clinically important slope and that the residual variation of serum concentration of 25(OH)D around the mean line was normally distributed. To demonstrate a dose-response relation (slope) in the range of 1.0–1.5 at $\alpha = 0.05$ with 90% power, a total of 105 children would need to complete the study ($n = 35$ in each of the 3 treatment groups). A slope of 2.2 has previously been reported in Finnish children (mean age: 9 y) (15), and a slope of 2.0–2.6 has been reported in Finnish and Danish girls (34). To take into account the 15–20% potential dropouts and blood samples with insufficient volumes, we enrolled a total of 130 children.

Statistical analyses

Because this was an efficacy study investigating the biological dose-response association between vitamin D intake and serum concentrations to inform dietary requirements for vitamin D, the primary analysis was carried out as a per-protocol analysis in which noncompliers, including those who went on vacation south of 51°N during the intervention or had a tablet compliance <80%, were excluded from the analyses. In addition, an intention-to-treat (ITT) analysis of serum 25(OH)D was performed of all 130 children who were initially randomly assigned, including both dropouts and noncompliers. For the ITT analysis, missing baseline values were imputed with the use of an ANCOVA model based on data that were available for all children at baseline, whereas missing dietary intake and serum 25(OH)D values at follow-up were imputed with the use of the baseline-observation-carried-forward method.

Differences between groups in biochemical measures and dietary intake of vitamin D and calcium after the intervention were analyzed with the use of mixed-effects ANCOVA models, with intervention group and baseline value as fixed effects and siblings as random effects. The effect of including other covariates one by one in the models of serum 25(OH)D, PTH, and calcium were also explored. Changes during the intervention in serum 25(OH)D, dietary vitamin D and calcium, and PTH within each group were investigated with the use of paired t tests. Differences in distributions of participants below and above various 25(OH)D cutoffs were examined with Pearson chi-square tests for contingency tables. Model assumptions were checked by inspecting residual and quantile-quantile plots, and vitamin D and calcium intakes were log-transformed before analysis to alleviate right skewness. Children included and not included in the analyses were compared on selected characteristics with 2-sample t tests for continuous variables and Pearson chi-square tests for categorical variables. Data were analyzed with the use of Stata version 14.0 (StataCorp LP), and $P < 0.05$ was considered statistically significant.

A series of models were assessed for best fit to the relation between total vitamin D intake and serum 25(OH)D at endpoint, and a curvilinear model was chosen $\{y = b_2 + b_0 \times [1 - \exp(-x/b_1)]\}$. The model was also validated by means of residual and quantile-quantile plots. The 95% prediction intervals of the required vitamin D intake were calculated to estimate the probable range of intakes in the target population. Moreover, the required vitamin D intakes to maintain 50%, 90%, 95%, and 97.5% of the children above serum 25(OH)D thresholds of 25, 30, 40, and 50 nmol/L were estimated from the chosen model by inverse regression on the lower limits of the prediction intervals. In addition, 95% CIs for the lower prediction limits were obtained with the use of bias-corrected bootstrap based on 1000 replications. These analyses were carried out with the use of R version 3.2.2 (22).

RESULTS

Subject characteristics, compliance, and blinding

As seen in **Table 1**, randomization was successful with regard to baseline characteristics. Children had a mean ± SD age of 6.6 ± 1.5 y, and 82% were of normal weight. About half of the children were girls, and only 4 had entered puberty (Tanner stage II). The children included in the per-protocol analyses (n = 119) had a median vitamin D intake of 1.8 μg/d (IQR: 1.2, 2.5 μg/d) and a mean ± SD serum 25(OH)D of 56.7 ± 12.3 nmol/L at baseline (range: 28.7–101.4 nmol/L). Dietary intakes of vitamin D and calcium did not change during the intervention in any of the groups ($P > 0.09$) and did not differ between groups at endpoint (**Table 2**). However, boys had a higher vitamin D intake than girls ($P = 0.053$ and $P = 0.0007$ at baseline and endpoint, respectively; data not shown). In total, 31% of the children had serum 25(OH)D <50 nmol/L, and 2% had concentrations <30 nmol/L at baseline (**Supplemental Table 1**).

Four children (3%) withdrew from the study because of an unwillingness to undergo blood sampling (Figure 1). In addition, 1 child who could not be successfully blood sampled at baseline, 4 children who went on vacation south of 51°N during the 20-wk intervention study, and 2 children with a tablet compliance <80% were excluded from the per-protocol analyses, resulting in a final sample size of 119. The 119 children did not differ from the 11 children who were not included with respect to age, sex, BMI-for-age z score, or parental education ($P > 0.15$).

TABLE 1
Baseline characteristics of the subjects in the 3 intervention groups[1]

	Placebo	10 μg/d	20 μg/d
n	43	44	43
Parental education, n (%)[2]			
≤14 y	6 (14)	8 (18)	7 (16)
15–16 y	12 (28)	11 (25)	8 (19)
≥17 y	25 (58)	25 (57)	28 (65)
Girls, n (%)	23 (53)	23 (52)	23 (53)
Age, y	6.5 ± 1.5	6.7 ± 1.5	6.7 ± 1.4
Height, cm	120.9 ± 10.8	122.7 ± 11.6	122.4 ± 10.0
Weight, kg	22.8 ± 5.0	23.8 ± 5.9	23.9 ± 4.9
BMI-for-age z scores[3]	−0.10 ± 0.97	−0.04 ± 0.78	0.14 ± 0.74
Waist circumference, cm	54.7 ± 4.7	55.0 ± 4.5	55.6 ± 4.5
Weight status, n (%)[4]			
Underweight	8 (19)	3 (7)	2 (5)
Normal weight	29 (67)	37 (84)	40 (93)
Overweight or obese	6 (14)	4 (9)	1 (2)

[1] Values are means ± SDs unless otherwise indicated; n = 130.
[2] Defined as highest level of education obtained in the household.
[3] Calculated with the use of WHO AnthroPlus software (31).
[4] Based on references 35 and 36.

Median tablet compliance was 96% (IQR: 93%, 99%) and did not differ between the 3 groups ($P = 0.62$). There were no associations between the group allocation of the child and the investigator or parent's guess on group ($P = 0.18$ and $P = 0.65$, respectively), suggesting that blinding was highly successful.

Effects of vitamin D supplementation on serum 25(OH)D, PTH, and calcium

A significant difference in serum 25(OH)D was seen between groups at endpoint (Table 2). Serum 25(OH)D increased by a mean ± SE of 4.9 ± 1.3 nmol/L ($P < 0.001$) and 17.7 ± 1.8 nmol/L ($P < 0.0001$) during the intervention in the 10- and 20-μg/d groups, respectively, and decreased by 24.1 ± 1.2 nmol/L ($P < 0.0001$) in the placebo group. In the ITT analysis ($n = 130$), serum 25(OH)D increased by a mean ± SE of 4.1 ± 1.2 and 16.5 ± 1.8 nmol/L in the 10- and 20-μg/d groups, respectively, and decreased by 23.5 ± 1.3 nmol/L in the placebo group ($P < 0.0001$). Age, sex, height, BMI-for-age z score, parental education, and tablet compliance were not significant in the model of serum 25(OH)D (data not shown).

Within each of the 3 intervention groups, changes in serum 25(OH)D during the intervention were dependent on serum 25(OH)D values at baseline ($P < 0.001$). In the placebo group, the children with the lowest values had the smallest decline in serum 25(OH)D, and in the 2 supplemented groups the largest increases in serum 25(OH)D were seen in those with the initially lowest values (data not shown).

At endpoint, 46% of the children in the placebo group were vitamin D-deficient (serum 25(OH)D <30 nmol/L), and none of the children in the placebo group had adequate status (>50 nmol/L) (Supplemental Table 1). In comparison, none of the children in the 2 supplemented groups was deficient after the winter period, whereas vitamin D adequacy was found at endpoint in 92% and 100% of the children in the 10- and 20-μg/d groups, respectively (Supplemental Table 1). None of the children had serum 25(OH)D ≥125 nmol/L at any time point.

Baseline-adjusted serum PTH was higher in the placebo group than the 2 vitamin D-supplemented groups at endpoint, whereas serum calcium concentrations did not differ between the groups (Table 2).

Dose-response relation between total vitamin D intake and serum 25(OH)D

Figure 2 shows the curvilinear relation between the total vitamin D intake (dietary and supplemental) and endpoint serum 25(OH)D in children aged 4–8 y. The model explained 77% of the variability around the mean in serum 25(OH)D. With the use of the lower 95% prediction interval, we estimated that the vitamin D intakes required to maintain winter serum 25(OH)D >25, >30, and >50 nmol/L in 97.5% of the children were 6.4, 8.3, and 19.5 μg/d, respectively (**Table 3**). The vitamin D intake required to maintain winter serum 25(OH)D >40 nmol/L in 50% of the children (i.e., the EAR) (4) was 4.4 μg/d (Table 3).

Safety and adverse events

None of the children had elevated serum calcium concentrations (>2.7 mmol/L) either at baseline or endpoint. In addition, no serious adverse events or adverse events related to the supplementation were reported either at midpoint or endpoint. The most commonly reported health change during the winter intervention period was being more ill with cold-related symptoms than during the summer months before the intervention; this was reported in

TABLE 2
Dietary intakes of vitamin D and calcium, serum 25(OH)D, plasma PTH, and serum calcium measured at baseline and endpoint[1]

	Placebo	10 μg/d	20 μg/d	P
n	41	38	40	
Dietary vitamin D, μg/d				
Baseline	1.8 (1.2, 2.5)[2]	1.6 (1.0, 2.2)	2.0 (1.5, 2.7)	
Endpoint	1.8 (1.3, 2.4)	1.5 (1.0, 2.6)	1.7 (1.4, 2.7)	1.00
Dietary calcium, mg/d				
Baseline	602 (426, 775)	714 (506, 829)	852 (506, 1022)	
Endpoint	560 (447, 759)	657 (472, 819)	701 (530, 848)	0.89
Serum 25(OH)D, nmol/L				
Baseline	55.2 ± 10.8[3]	56.9 ± 12.7	58.1 ± 13.5	
Endpoint	31.1 ± 7.5[a]	61.8 ± 10.6[b]	75.8 ± 11.5[c]	<0.0001
Plasma PTH, pmol/L				
Baseline[4]	1.77 ± 0.95	2.10 ± 0.82	2.04 ± 1.24	
Endpoint[5]	2.52 ± 1.23[a]	2.20 ± 0.73[b]	1.91 ± 0.85[b]	<0.0001
Serum calcium,[6] mmol/L				
Baseline	2.41 ± 0.07	2.40 ± 0.07	2.41 ± 0.07	
Endpoint	2.13 ± 0.08	2.12 ± 0.08	2.15 ± 0.07	0.45

[1] P values for differences between groups at endpoint by ANCOVA adjusted for baseline and with siblings as a random effect. Values in the same row but with different superscript letters are significantly different ($P < 0.05$), $n = 119$. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
[2] Median; IQR in parentheses (all such values).
[3] Mean ± SD (all such values).
[4] $n = 41$, 37, and 37 in the 0, 10, and 20-μg/d groups, respectively.
[5] $n = 39$, 36, and 37 in the 0, 10, and 20-μg/d groups, respectively.
[6] Albumin-corrected calcium values.

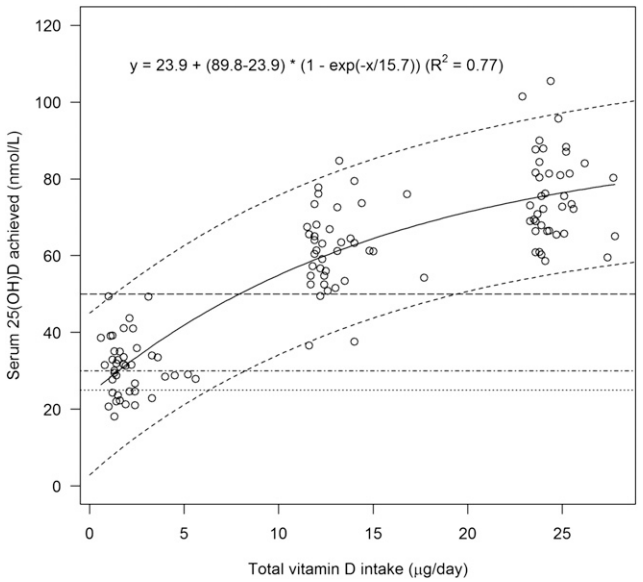

FIGURE 2 The relation between achieved serum 25(OH)D concentrations in late winter and total vitamin D intake in healthy children aged 4–8 y living at 55°N. Mean response is indicated by the central line, and the outer lines are its 95% prediction intervals, n = 119. Horizontal hashed lines represent serum 25(OH)D thresholds of 25, 30, and 50 nmol/L, respectively. 25(OH)D, 25-hydroxyvitamin D.

17%, 17%, and 14% of the children in the placebo and 10- and 20-μg/d groups, respectively.

DISCUSSION

This report of a double-blind dose-response vitamin D trial in a well-characterized sample of healthy young children in Denmark has identified the dietary requirement for vitamin D to maintain wintertime serum 25(OH)D >25, >30, and >50 nmol/L in 97.5% of children aged 4–8 y as 6.4, 8.3, and 19.5 μg/d, respectively. We conducted this study with the use of the same design protocol previously implemented in studies of young and old adults (18, 19), which stipulated requirements of ~10 and 25 μg/d to maintain serum 25(OH)D >30 and >50 nmol/L, respectively. Our estimates are only modestly different from those reported in Finnish and Danish adolescent girls by Cashman et al. (10.3 and 18.6 μg/d for >30 and >50 nmol/L, respectively), albeit with a slightly modified design (34, 37). This high level of agreement between trial data provides firm evidence that at high latitudes (>51°N), 8–10 μg/d is required to maintain serum 25(OH)D >30 nmol/L during the winter. This is in line with the current recommendation of 10 μg vitamin D/d for white children and adults in Nordic countries (6) as well as the United Kingdom (5). The dietary requirement to achieve a serum 25(OH)D of 40 nmol/L in 50% of the children aged 4–8 y [i.e., the EAR value as defined by the IOM (4)] was 4.4 μg/d, which is slightly lower than the EAR of 6.3 μg/d reported in adolescent girls (34) and similar to the value of 4.1 μg/d in adults (18). These requirements are all somewhat lower than the EAR value of 10 μg/d set by the IOM for children aged 4–8 y (4). In relation to the Recommended Dietary Allowance value, based on a serum 25(OH)D concentration of 50 nmol/L, the 19.5-μg/d estimate for this study is slightly lower than that reported previously for younger and older adults at ~25 μg/d (18, 19). This suggests that the dietary requirement for vitamin D is, at least to some extent, influenced by age and/or body size.

In this study, the vitamin D stores accumulated during the preceding summer in the placebo group of young children ensured a mean serum 25(OH)D concentration above the deficiency cutoff by the end of winter. Nevertheless, nearly half of the children in this nonsupplemented group were vitamin D–deficient in February and March. Moreover, PTH concentrations increased in the placebo group during winter, whereas vitamin D supplementation inhibited this elevation, as also seen previously by others (38). This may be a concern because low serum 25(OH)D and an associated increase in PTH have been linked to increased bone turnover (39). However, the exact implications of recurrent low winter vitamin D status and the associated elevation of PTH concentrations are uncertain in children, in which high and normal PTH concentrations have been linked to higher bone growth (40).

Although a dose-response relation occurred with vitamin D supplementation, the difference in serum 25(OH)D between the placebo and 10-μg/d groups at endpoint was more than twice as large as the difference between the 2 supplemented groups. This was confirmed by the curvilinear relation between vitamin D intake and serum 25(OH)D and could indicate that saturation in serum 25(OH)D may appear at higher vitamin D intakes, as suggested by others (41) and the IOM (4), potentially because of

TABLE 3
Estimated dietary requirements for vitamin D to maintain serum 25(OH)D above selected concentrations in children aged 4–8 y during winter[1]

	Percentile, μg/d (95% CI)			
Serum 25(OH)D, nmol/L	50th[2]	90th	95th	97.5th[3]
>25	0.3 (0, 1.1)	4.0 (3.1, 5.0)	5.2 (4.2, 6.6)	6.4 (5.2, 7.9)
>30	1.6 (0.6, 2.4)	5.6 (4.5, 6.8)	7.0 (5.7, 8.4)	8.3 (6.9, 10.2)
>40	4.4 (3.6, 5.3)	9.4 (7.9, 11.0)	11.2 (9.5, 12.9)	12.9 (11.0, 15.1)
>50	8.0 (6.8, 9.2)	14.5 (12.6, 16.4)	17.0 (15.0, 19.1)	19.5 (17.2, 23.0)

[1] Results based on a nonlinear model of serum 25(OH)D concentration as a function of vitamin D intake $\{y = b_2 + b_0 \times [1 - \exp(-x/b_1)]\}$, n = 119; 95% CIs for the lower prediction limits were obtained with the use of bias-corrected bootstrap based on 1000 replications. 25(OH)D, 25-hydroxyvitamin D.

[2] The vitamin D intake that will maintain serum 25(OH)D concentrations in 50% of children aged 4–8 y above the indicated cutoff concentration during winter, representing an Estimated Average Requirement.

[3] The vitamin D intake that will maintain serum 25(OH)D concentrations in 97.5% of children aged 4–8 y above the indicated cutoff concentration during winter, representing a Recommended Dietary Allowance.

the saturation of the 25-hydoxylase enzyme, which converts vitamin D_3 to 25(OH)D in the liver (41). However, the plateau seen in this study appeared at lower intakes than the plateau seen in the studies in adults referred to by the IOM (4), which may support potential age differences. In line with this, having a lower baseline vitamin D status blunted the seasonal decline in serum 25(OH)D in the placebo group of this study, whereas within the supplemented groups those children with the highest initial serum 25(OH)D had the smallest increases in serum 25(OH)D during the intervention. The phenomenon of a blunted winter decline in serum 25(OH)D with lower baseline status is in accordance with other studies in children and adults (16, 18, 19, 34) and may indicate that children's vitamin D metabolism adapts to vitamin D scarcity.

This study is the first to our knowledge to present as a primary outcome the dietary vitamin D requirement for children aged <8 y living in northern latitudes. Strengths include the dose-response placebo-controlled winter design, which excluded dermal vitamin D synthesis and was specifically aimed at assessing dietary vitamin D requirements of young children. Moreover, we used 95% prediction intervals based on individual data rather than CIs in the dose-response relation between vitamin D intake and serum 25(OH)D for defining requirements. Prediction intervals take into account the interindividual variability in addition to sampling variation in the intake required to reach a specific 25(OH)D cutoff (17). Thus, prediction intervals provide the most likely range (95%) from which to expect future data points from the same target population and are appropriate for defining requirements. This study ensured efficient blinding of investigators and parents, high tablet compliance, and a low dropout rate and was well powered. The dietary intake of vitamin D used in the mathematical modeling was estimated with the use of a previously validated FFQ specifically developed for quantifying dietary intakes of vitamin D and calcium (26). This method of food item quantification is suitable for food items not eaten on a daily basis, such as fish, which is one of the main vitamin D sources in the Danish population. Finally, serum 25(OH)D was analyzed by LC-MS/MS, the gold standard for analyzing 25(OH)D (42). Because no cases of hypercalcemia or adverse events related to the supplementation were found, the supplementary vitamin D doses used were found to be safe, as expected.

The presented biological dose-responsiveness of serum 25(OH)D to intake of vitamin D_3 is likely to be extendable to most other populations of white children living in northern latitudes, whereas the dose-response curve and dietary vitamin D requirements of other ethnic groups or overweight and obese children should be investigated separately in other studies. A previous dose-response trial in African American and white children aged 9–13 y at 34°N and 40°N in the United States did not find differences in the rate of increase in serum 25(OH)D between the 2 ethnic groups (43). On the other hand, a trial that compared the serum 25(OH)D response of obese and nonobese African American children reported different treatment responses according to weight status (44). It is also possible that the curvilinear response of serum 25(OH)D to vitamin D intake in our study, which required a nonlinear model to be fitted in comparison to the linear model applied in the study in adolescent girls (34), may have affected the model estimates of the vitamin D intake required to reach a serum 25(OH)D concentration of 50 nmol/L, at which response was more blunted.

In conclusion, vitamin D intakes between 8 and 20 μg/d are required by white children aged 4–8 y to maintain serum 25(OH)D concentrations >30–50 nmol/L throughout the winter in northern latitudes, with the exact requirement dependent on the serum 25(OH)D threshold chosen. Moreover, the intake required to maintain serum 25(OH)D >40 nmol/L in 50% of the participants is 4 μg/d. These data may contribute to the continuing refinement of requirements of vitamin D in young children.

The authors' responsibilities were as follows—CTD, SAL-N, TJS, KDC, MK, and C Mølgaard: designed the study; C Mortensen, CTD, HH, and C Mølgaard: conducted the research; C Mortensen, CR, and KDC: performed the statistical analyses; AH: checked the quality of the dietary assessments and supervised the dietary calculations; KD: performed the LC-MS/MS analyses; C Mortensen and CTD: drafted the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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