Osteoporos Int (2016) 27:2459–2466
DOI 10.1007/s00198-016-3549-z

ORIGINAL ARTICLE

Vitamin D supplementation in breastfed infants from Montréal, Canada: 25-hydroxyvitamin D and bone health effects from a follow-up study at 3 years of age

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Received: 30 October 2015 / Accepted: 22 February 2016 / Published online: 11 March 2016

Abstract
Summary Whether infant vitamin D supplementation may have long-term bone benefits is unclear. In this study, breastfed infants who received vitamin dosages greater than 400 IU/day did not have higher bone mineralization at 3 years. This study provides important data to inform pediatric public health recommendations for vitamin D.
Introduction North American health agencies recommend breastfed infants should be supplemented with 400 IU of vitamin D/day to support bone health. Few studies examined the long-term benefits of early life vitamin D supplementation on bone mineralization. The objective of this study was to determine if a dose-response relationship exists between infant vitamin D supplementation, vitamin D status, and bone outcomes at 3 years of age.
Methods This was a double-blind randomized trial of 132, 1-month-old healthy, breastfed infants from Montréal, Canada, between 2007 and 2010. In this longitudinal analysis, 87 infants (66 %) returned for follow-up at 3 years of age, between 2010 and 2013. At 1 month of age, participants were randomly assigned to receive oral cholecalciferol (vitamin D_3) supplements of 400, 800, 1200, or 1600 IU/day until 12 months of age. Lumbar spine vertebrae 1–4 (LS) bone mineral density (BMD), LS and whole body bone mineral content (BMC), and mineral accretion were measured by dual-energy x-ray absorptiometry at 3 years.
Results At follow-up, the treatment groups were similar in terms of diet, sun exposure, and demographics. There were no significant differences among the groups in LS or whole body BMC, BMD, or accretion. Although, 25(OH)D concentrations were not different among the groups, higher doses (1200 and 1600 IU/day) achieved higher 25(OH)D area under the curve from 1 to 36 months vs. 400 IU/day.
Conclusions This is the first longitudinal follow-up of an infant vitamin D dose-response study which examines bone mineralization at 3 years of age. Dosages higher than 400 IU/day do not appear to provide additional benefits to the bone at follow-up. Larger studies with more ethnically diverse groups are needed to confirm these results.

Keywords 25-hydroxyvitamin D · Bone mineral · Bone mineral accretion · Dual-energy x-ray absorptiometry · Infants · Pediatrics · Supplementation · Vitamin D

Trial registration clinical trials.gov Identifier NCT00381914

Electronic supplementary material The online version of this article (doi:10.1007/s00198-016-3549-z) contains supplementary material, which is available to authorized users.

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Introduction

The foremost functions of vitamin D are the regulation of calcium homeostasis and bone mineral metabolism [1], both of which are of particular importance to growing children. However, the value of circulating 25-hydroxyvitamin D (25(OH)D) concentrations for optimal health is not clear [2]. Health guidelines recommend a healthy range for 25(OH)D between 50 and 125 nmol/L (20–50 ng/ml) and a standard-of-care supplemental vitamin D intake of 400 IU/day for infants until provided through the diet [1, 3, 4]. To date, there have been few infant trials examining high dose vitamin D supplementation [5–7] and no study establishing long-term benefits on bone health.

The most accelerated rates of growth and bone mineral accretion occur during infancy [8]; maximizing accretion during early life has been suggested to provide later benefits to the bone [9, 10]. Vitamin D status positively associates with bone mass in infants and children [11–14] but some infant trials show no relationship [6, 15, 16] suggesting benefits may not readily appear. Specifically, positive relationships exist for vitamin D exposure *in utero* [11] and in infancy [17] with later bone mass in childhood. Prepubertal girls with moderate vitamin D status had significantly higher cortical radial and tibia volumetric bone mineral density (BMD) as well as lower bone resorption and parathyroid hormone (PTH) than those with deficiency (≤25 nmol/L 25(OH)D) [18]. In a cross sectional analysis of preschool age children, we observed that whole body bone mineral content (BMC) and BMD were positively linked to having 25(OH)D over 75 nmol/L [19]. However, there continues to be limited data regarding the impact of vitamin D status on bone mineral accretion in early life, requiring longitudinal follow-up.

Recently, we published a vitamin D dose-response study (NCT00381914) of daily vitamin D_3 supplementation ranging from 400 to 1600 IU in breastfed infants from 1 to 12 months of age. As previously reported [5], all dosages supported 25(OH)D_3 concentrations above 50 nmol/L after 2 months of supplementation; however, no significant benefits to bone mineral accretion were observed among the groups by 1 year of age. In the present study, which is a longitudinal follow-up from our study published in 2013 [5], we tested the possibility that dose-response effects of vitamin D given in infancy would be observed in bone health later in childhood. As growth in infancy sets the foundation for subsequent skeletal growth [20] and in agreement with previous studies conducted in prepubertal children [14, 17], we hypothesized that in the context of sustained healthy vitamin D status, bone health targets achieved in infancy would continue to 3 years of age. Additionally, the role of vitamin D epimers and metabolites in childhood is not well understood, and thus, secondary outcomes of this study included exploring vitamin D metabolism at 3 years.

Materials and methods

The design and main outcomes for the original study are previously described in detail [5]. Briefly, 132 healthy, term, breastfed (consuming ≥80 % of total milk volume) infants were enrolled between 2007 and 2010 from greater Montréal (Québec, Canada), located at 45°N. At 1 month of age (baseline), infants were randomized to receive one of four dosages of oral cholecalciferol (400, 800, 1200, or 1600 IU/day of vitamin D_3) and were monitored at study visits conducted at 3, 6, 9, and 12 months of age. The 1600 IU/day group was discontinued early in the study due to plasma 25(OH)D_3 concentrations of 250 nmol/L or greater by 3 months of age based on an enzyme immunoassay (see [5] for stopping rules). On average, participants were supplemented with the study vitamin D for 11 months, as reported compliance with supplementation ranged between 84 and 90 % over the course of the trial. At the final trial visit, completed at 12 months of age, all parents regardless of the infant treatment group were advised about dietary sources of vitamin D and encouraged to provide a commercially available supplement of 400 IU/day (based on IOM recommendation prior to 2011), if they were unable to get this amount through their diet. The present observational follow-up was conducted when the children were 36 months (3 years) of age, between 2010 and 2013. McGill University and George Mason University institutional review boards provided ethical approval. Parents self-identified race/ethnicity, using Canadian Census criteria, and demographics including education and income were collected.

Anthropometry

Height was measured (nearest mm) using a stadiometer (Seca 213, Seca Medical Scales and Measuring Systems) and weight using a calibrated scale (Detecto) with the child wearing light clothing and no shoes. BMI was calculated as kg/m^2, and weight-for-age (WAZ), height-for-age (HAZ), and BMI-for-age (BAZ) Z-scores were derived based on the World Health Organization 2007 growth standards (WHO AnthroPlus) [21].

Laboratory analysis

A non-fasted capillary blood sample (lithium heparin) in addition to a spot urine sample was collected. To assess calcium homeostasis, blood-ionized calcium (ABL 725 series blood gas analyzer; Radiometer America), and plasma total calcium, phosphorus, alkaline phosphatase, and urinary calcium:creatinine were measured (Beckman Coulter DXC600) immediately with intra-assay coefficient of variation (CV) of <5 %. Additional plasma was stored at −80 °C for batch analysis. Plasma intact PTH was measured using an ELISA (Immutopics International) sensitive to 12 pg/mL (12 ng/L) and CV <8 %. Plasma C-terminal telopeptide of

type I collagen (CTX), a biomarker of bone turnover, was measured using CrossLaps® ELISA (Immunodiagnostic Systems Ltd) sensitive to 0.020 ng/mL and CV 9.4 %. We have previously noted high proportions of both 3-epimer-25(OH)D (3-epi-25(OH)D_3) and 24,25-dihydroxyvitamin D (24,25$(OH)_2D_3$) in relation to plasma 25(OH)D_3 [22]; thus, in order to further explore the relationship between vitamin D metabolism and bone accretion, plasma 3-epi-25(OH)D_3, 24,25$(OH)_2D_3$ and 25(OH)D_3 were quantified by Warnex Bioanalytical Services using liquid-chromatography tandem mass spectrometry (LC-MS/MS) [23]. See [5, 22] for method description. The intra-assay CVs were <15 % for all vitamin D metabolites; the laboratory was certified by the Vitamin D External Quality Assessment Scheme. The measured 25(OH)D_3 concentrations of the National Institute of Standards and Technology (NIST) standard reference materials (SRM 968e) [24] were within 7.0 and 2.5 % of the certified values for levels 2 and 3, respectively. The ability to achieve plasma 25(OH)D concentrations of ≥50 and ≥75 nmol/L was explored as both are considered health targets [3, 4, 25].

Dietary data

Dietary intake was measured using three 24-h recalls (one in person, two by telephone), and nutrient intake was generated using Nutritionist Pro software version 4.7.0 (Axxya Systems LLC) using the 2010b Canadian Nutrient File database (Health Canada). The proportion of children achieving the estimated average requirement (EAR; 400 IU/day) and recommended dietary allowance (RDA; 600 IU/day) was assessed. In addition, a validated food frequency questionnaire (FFQ) for preschool children, completed by the primary caregiver, was used to capture calcium and vitamin D intake over the past month [26]. Total dietary and supplemental vitamin D intake was categorized into tertiles. Cows' milk and margarine are mandated to be fortified with vitamin D in Canada [27], as natural dietary sources of vitamin D are limited. Fortified cow's milk contributes 66–72 % of total vitamin D intake to Canadian children's diet [28].

Sun exposure

Questionnaires were used to assess sun exposure, winter travel, and use of sunscreen for the 2 years prior to the follow-up visit. Sun exposure was collected retrospectively as a percentage of body surface area (BSA) exposed, frequency of sunscreen use, and total hours spent in direct sunlight per day. Sun index was calculated for each child by multiplying the percent BSA exposed by the time spent outside (minutes per day) [29]. This index does not consider the use of sun block. Season of visit was defined using the equinox and solstice dates [30].

Skin pigmentation

Skin pigmentation was measured on the constitutive upper underarm and facultative forehead, forearm, and outer lower leg using a portable computerized spectrophotometer (CM-600D, Konica Minolta). Based on the Commission Internationale de l'Eclairage colorimetry system (L*a*b*), the individual typological angle (ITA) {ITA° = [arc tangent (L* − 50) / b*)] 180/3.14159} of the upper underarm site was calculated [31]. Infants were classified into five skin phototypes as follows: dark (≤10°), olive (10–28°), medium (28–41°), fair (41–55°), and very fair (>55°).

Bone parameters

Bone area, bone mineral content (BMC), and areal bone mineral density (aBMD) of the whole body and L1 to L4 vertebrae were measured using dual-energy x-ray absorptiometry (DXA; QDR 4500A, APEX software version 13.2.3 or 13.3.3, Hologic Inc., Waltham, MA). Children were scanned according to International Society for Clinical Densitometry (ISCD) guidelines [32]; whole body was scanned using infant whole body software between 0–12 months and adult whole body at 36 months, whereas lumbar spine scans were captured using anterior-posterior software (express mode at 36 months). Values are expressed as absolute BMC, aBMD, Z-scores (reference: Pediatric, Hologic 2005 [33]), % accretion (i.e., Δ BMC from 36–1 month/BMC at 1 month × 100 %) as well as accretion/year (i.e., Δ BMC from 36–1 month/(days in period/365 days/year)). Whole body BMC was further corrected for weight, height, and lean mass as suggested by the ISCD [32]. The CV was 1 % for BMC and 0.3 % for BMD using a spine phantom (Hologic phantom No. 14774).

Statistical methods

Baseline differences among the groups were tested using analysis of variance (ANOVA) and X^2. The mean area under the curve (AUC) for 25(OH)D concentrations across all time points was calculated based on the trapezoidal method. The effects of vitamin D treatment on plasma vitamin D metabolite and epimer concentrations, AUC, anthropometry, biomarkers of bone metabolism, and bone mineral accretion were explored and tested using mixed-model ANOVA analyzed as intent-to-treat. Participants with missing data are not dropped using this procedure. Post hoc tests (estimates statements) were used to test for differences between the treatment groups and Bonferroni adjustment for multiple comparisons applied. Data analysis was conducted both including and excluding the 1600 IU/day group ($n = 11$) with similar results; therefore, all the groups were

included in the final analysis. Using an alpha level of 0.05 for a two-tailed test and 90 % power, a sample size of 25 subjects/group would detect a 5 % difference in whole body BMD (0.56 to 0.59 g/cm^2). Statistical significance for all the tests was set at $p < 0.05$ after adjustment for multiple comparisons where applicable. Values reported are in mean [95 % CI] or number (%).

Results

From the original 132 participants in the trial, 66 % (49 boys and 38 girls) returned for the 3-year follow-up (average 36.7 months). This represented 87 % of those who completed the original study (85 of 98) and 2 drop-outs, which returned for follow-up (Online Resource Fig. 1). All children were healthy, and four (5 %) children were categorized as >2 SD based on BAZ. Except for age being greater in the 1600 IU group, there were no differences among the groups in demographic, growth, nutrition, and sun exposure factors at 3 years (Table 1). There were no differences in the treatment group allocation among returners vs. non-returners; however, mothers of returners were older ($p = 0.05$), typically white ($p = 0.01$) and differed by season of sampling ($p = 0.04$) than those of non-returners. There were no significant differences in 25(OH)D concentrations or status at 3 years between returners and non-returners (Online Resource Table 1).

No differences among the vitamin D treatment groups were noted for BMC, BMD, or BMD Z-score of the LS or whole body (Table 2). In line with previous results [19], sex did not affect bone outcomes; thus, data are presented combined. No significant difference in lumbar spine or whole body body mineral accretion among the groups was noted. There were no effects of treatment or vitamin D status at 3 years on biomarkers related to the bone including ionized calcium (mean 5.16, 95 % CI 5.08–5.20 mg/dL), plasma CTX (1.13, 1.06–1.20 ng/mL), or PTH (34.4, 31.8–37.1 pg/mL) at 3 years.

At 3 years, the mean plasma 25(OH)D_3 for all the children was 73.6 nmol/L, 25(OH)D_3:24,25(OH)$_2$D ratio was 8:1, and 25(OH)D_3:3-epi-25(OH)$_2D_3$ was 20:1. Plasma 25(OH)D_3, 24,25(OH)$_2D_3$, and 3-epimer-25(OH)D_3 were not different among the groups (Fig. 1a). Significant differences were noted between the 1200 and 1600 IU/day vs. 400 IU/day in 25(OH)D AUC from 1 to 36 months ($p < 0.01$) (Fig. 1b). Of all the children at 3 years, none were below 30 nmol/L, 1 % ($n = 1$ of 87) fell below 40 nmol/L, 10 % ($n = 9$) below 50 nmol/L, and 62 % ($n = 54$) below 75 nmol/L of 25(OH)D. This reflects the following changes in 25(OH)D from 1 month of age: 6 % ($n = 4$ of 65) below 30 nmol/L, 17 % ($n = 11$) below 40 nmol/L, 29 % ($n = 19$) below 50 nmol/L, and 72 % ($n = 47$) below 75 nmol/L of 25(OH)D. At 3 years, the proportion of children achieving the 50 or 75 nmol/L cut-off was not statistically different among the groups (Fig. 1c).

Discussion

This is the first follow-up study to explore bone health outcomes at 3 years of age in an infant cohort randomized to receive vitamin D supplementation between 400 and 1600 IU/day from 1 to 12 months of age. In the present follow-up of previously breastfed infants, receipt of more than 400 IU of vitamin D/day did not appear to provide additional benefits for bone mineralization at 3 years of age, thus providing support for our current infant vitamin D health policy recommendations [1, 3] in the amount of 400 IU/day. Overall, children at 3 years were all healthy, growing and consuming an adequate diet, with no differences among the groups. Macronutrient intakes were in line with the acceptable macronutrient distribution ranges [34]; dietary calcium intakes were above the RDA of 700 mg/day; however, vitamin D intake from diet alone was unable to meet the RDA of 600 IU/day in all the children.

In a previous retrospective analysis [17], girls receiving an infant vitamin D supplement of 400 IU/day over a 12-month period (range 2–48 months) had a 6 % higher BMD of the distal radius and 9 % higher femoral neck at 7–9 years, after adjustment for height, implying supplementation during infancy may have long-term bone benefits. A recent meta-analysis [35] reported vitamin D supplementation during childhood led to improvements in the bone in a sub-group of vitamin D-deficient children (25(OH)$D_3 < 35$ nmol/L) but not in healthy children. The lack of dosage effect on bone outcomes in the present sample of children may be attributed to the robust baseline plasma 25(OH)D concentrations at 1 month of age, with an average of 56 nmol/L (95 % CI, 56–67 nmol/L) and very few (6 %) below 30 nmol/L. BMD values for this group of children were above average with BMD Z-score for the lumbar spine of 0.4 and whole body of 2.0. It is unclear whether the higher whole body Z-scores observed in the present study reflect differences in equipment models, scan modes or software versions, or population characteristics compared to the reference [33] or true differences in bone density. Discrepancies among pediatric reference data have been previously observed [36–38] with larger variation due to younger age [38].

These data add to our knowledge on the biological activity of vitamin D metabolites and epimers on pediatric bone. Although the function of 3-epi-25(OH)D_3 is unknown, our study [5, 22] as well as others [39] have shown the 3-epi-25(OH)D_3 to be high during the first year of life (95 % CI 19.8–22.9 nmol/L) [5] and decline by 3 years (95 % CI 2.7–4.0 nmol/L). The value of 24,25(OH)$_2D_3$ stayed fairly

Table 1 Characteristics at 36 months by the treatment group. Data are mean [95 % CI] for continuous or number (%) for categorical variables

Characteristics	Vitamin D_3 supplementation dosage				*P* value
	400 IU/day ($n=25$)	800 IU/day ($n=25$)	1200 IU/day ($n=26$)	1600 IU/day ($n=11$)	
Sex					0.996
% male	48.0	64.0	57.7	54.6	
Age, months	36.6[a] [36.3–36.8]	36.6[a] [36.2–37.0]	36.5[a] [36.3–36.8]	37.9[b] [36.6–39.2]	0.006
Skin color					0.716
% very fair	68.0	56.0	76.9	70.0	
% fair	16.0	28.0	19.2	20.0	
% medium	4.0	4.0	3.9	10.0	
% olive	12.0	8.0	0	0	
% dark	0	4.0	0	0	
Weight-for-age Z-score	0.37 [0.06–0.68]	0.06 [−0.30–0.43]	0.50 [0.15–0.85]	0.43 [0.26–1.12]	0.314
% within 2 SD	100	100	96	91	
Height-for-age Z-score	−0.12 [−0.44–0.19]	−0.27 [−0.70–0.16]	−0.21 [−0.52–0.11]	0.14 [−0.51–0.78]	0.628
% within 2 SD	100	96	100	100	
BMI-for-age Z-score	0.63 [0.30–0.97]	0.32 [0.01–0.63]	0.84 [0.50–1.18]	0.50 [−0.03–1.02]	0.138
% within 2 SD	96	100	92	91	
Sun exposure					0.958
Sun index, h/week exposed % BSA	1.63 [1.39–1.87]	1.61 [1.32–1.90]	1.63 [1.37–1.90]	1.69 [1.28–2.11]	
Dietary intake, 24-h recalls (3 days)					
Total energy, kcal/day	1434 [1302–1565]	1394 [1279–1510]	1502 [1355–1650]	1367 [1118–1616]	0.580
Protein, % total energy/day	16.2 [15.0–17.4]	15.8 [14.6–17.1]	15.2 [13.9–16.6]	18.1 [15.7–20.4]	0.100
Carbohydrate, % total energy/day	57.0 [54.0–60.0]	54.3 [51.4–57.2]	55.9 [53.2–58.6]	51.0 [29.8–34.8]	0.086
Fat, % total energy/day	28.8 [26.2–31.3]	31.1 [28.8–33.3]	30.8 [28.9–32.8]	32.3 [29.8–34.8]	0.227
Calcium, mg/day	970 [829–1111]	967 [847–1087]	931 [792–1069]	965 [709–1122]	0.972
Vitamin D, IU/day (food only)	247 [202–292]	280 [227–333]	285 [221–348]	302 [92–512]	0.756
% achieved EAR (400 IU/day)	5	14	29	11	0.216
Vitamin D, total diet and supplements, food frequency questionnaire (past 30 days)					0.136
Tertile 1: % ≤ 369 IU/day	40.0	25.0	19.0	36.0	
Tertile 2: % 369–534 IU/day	16.0	42.0	46.0	9.0	
Tertile 3: % ≥ 534 IU/day	44.0	33.0	35.0	55.0	

Continuous variables tested by ANOVA and categorical by X^2 (Fisher's exact). Significant differences among the groups reported as different superscript letters

constant from 12 months (95 % CI 9.1–10.9 nmol/L) to 3 years (95 % CI 11.0–17.0 nmol/L). Circulating levels of 3-epi-25(OH)D_3 may reflect vitamin D intake, which concurs with cell culture and animal studies [40], as 12-month-old infants in our dose-response trial were receiving between 42 and 166 IU/kg/day vs. 3-year olds ~33 IU/kg/day of vitamin D from both diet and supplements. In addition, the high concentrations of 25(OH)D and 3-epi-25(OH)D_3 observed in some infants during infancy [22] do not appear to adversely affect bone or PTH at 3 years as all the groups had similar absolute BMC and BMD.

The current sample at 3 years may not have been representative of our initial sample, although 66 % returned for follow-up, maternal characteristics differed in returners, and statistical analysis of imbalance was not performed to account for loss at follow-up. Our population studied was underrepresented in

Table 2 Bone outcomes of participants at follow-up by the treatment group. Data are mean [95 % CI] (*n*)

	Vitamin D_3 supplementation dosage				*P* value[a]
	400 IU/day	800 IU/day	1200 IU/day	1600 IU/day	
Lumbar spine, L1–L4					
BMC, g	11.5 [10.8–12.2] (25)	11.4 [10.8–12.0] (24)	11.8 [11.3–12.3] (25)	11.6 [10.3–12.9] (11)	0.865
BMD, g/cm^2	0.474 [0.450–0.500] (25)	0.463 [0.445–0.481] (24)	0.479 [0.462–0.496] (25)	0.463 [0.429–0.498] (11)	0.655
BMD Z-score	0.46 [0.05–-0.97]	0.18 [−0.22–-0.59]	0.62 [0.25–0.98]	−0.18 [−0.56–0.92]	0.457
% within 2 SD	84	92	92	100	
% within 3 SD	100	100	96	100	
Δ BMC 36–12 months, g	5.71 [4.96–6.47] (24)	5.74 [4.55–6.93] (24)	6.13 [5.61–6.65] (24)	5.89 [4.84–6.95] (11)	0.882
%	106 [87–124]	113 [86–138]	113 [98–129]	105 [85–124]	0.895
Δ BMC 36–1 months, g	8.48 [7.82–9.14] (25)	8.83 [8.29–9.37] (24)	9.00 [8.36–9.65] (25)	8.62 [7.47–9.76] (11)	0.652
%	285 [259–310]	368 [314–422]	351 [292–411]	306 [244–367]	0.052
Whole body					
BMC, g	600.6 [578.9–622.3] (24)	593.1 [575.4–610.7] (23)	593.1 [576.5–609.6] (25)	633.1 [591.5–674.9] (10)	0.110
BMD, g/cm^2	0.622 [0.607–0.637] (24)	0.626 [0.611–0.642] (23)	0.618 [0.604–0.633] (25)	0.651 [0.615–0.686] (10)	0.133
BMD Z-score	2.00 [1.53–2.47]	1.97 [1.58–2.36]	1.83 [1.46–2.19]	2.61 [1.63–3.59]	0.245
% within 2 SD	50	57	56	40	
% within 3 SD	79	87	88	60	
Δ BMC 36–12 months, g	354.2 [329.8–378.6] (18)	362.10 [345.3–378.9] (21)	361.7 [346.0–377.5] (21)	388.7 [333.4–444.1] (6)	0.377
%	150 [133–166]	159 [145–173]	159 [146–171]	156 [137–174]	0.748
Δ BMC 36–1 months, g	497.0 [474.6–519.5] (24)	496.6 [479.0–514.2] (23)	493.5 [475.3–511.8] (25)	534.0 [492.0–576.0] (10)	0.931
%	499 [448–550]	537 [482–591]	507 [467–547]	551 [474–630]	0.498

[a] Variables tested by ANOVA, not adjusted for multiple comparison

participants with darker skin pigmentation, who are known to be at higher risk of deficiency [1, 4]. A larger sample size would have allowed us to eliminate the chance of type II error. Some measures (i.e., sun exposure) may be subject to recall bias, and parental knowledge about the vitamin D dosage group may have influenced the results. Further, other technologies including peripheral quantitative computed tomography (pQCT) would allow the assessment of bone geometry in addition to BMC and BMD [41]. In order to verify our findings, it would be desirable to measure trabecular vs. cortical BMD by pQCT [6, 41] allowing detection of changes to different bone compartments. Lastly, regarding our biochemical indicators, no significant effects were noted along with bone mass, in line with a previous infant vitamin D supplementation trial [6]. Some markers may be sensitive to diurnal variation and feeding [42, 43] which were not controlled for in the study design.

In conclusion, these are the first results from an infant vitamin D supplementation trial to explore bone health effects on children at 3 years of age. This study supports our current

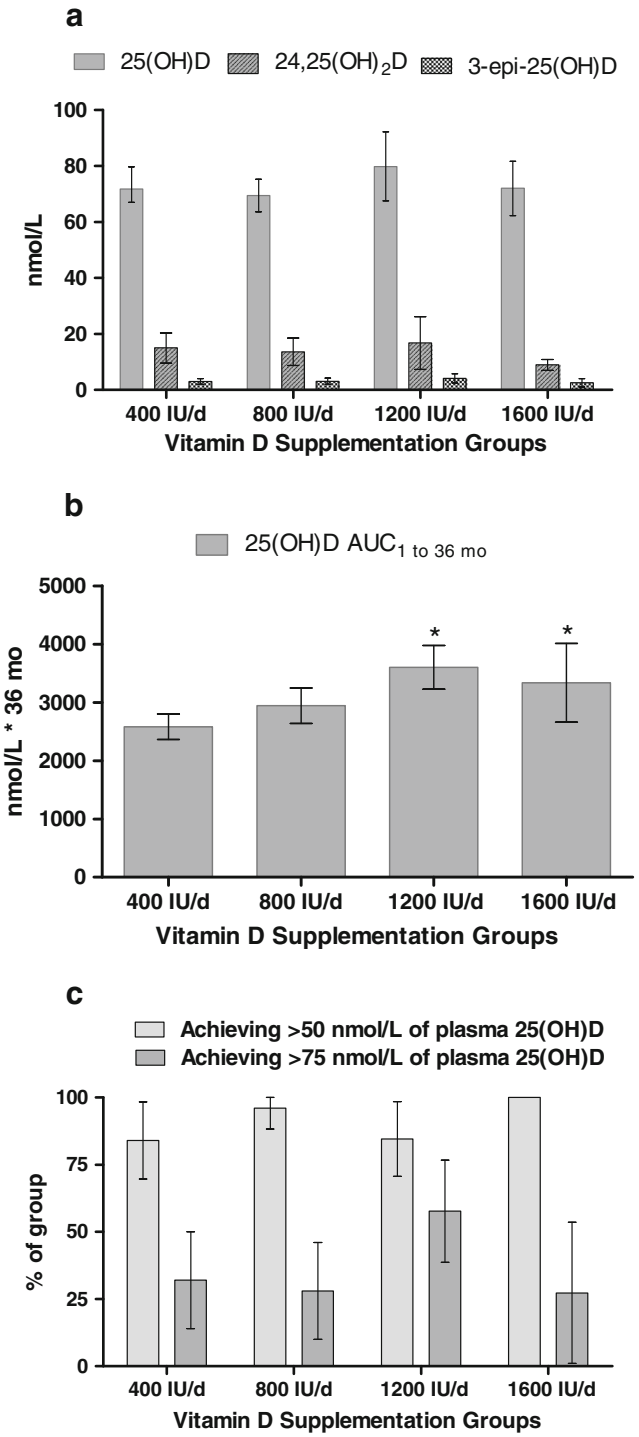

Fig. 1 Data are mean, and *bars* indicate 95 % CIs. *$p < 0.05$ vs. 400 IU/day. **a** Plasma $25(OH)D_3$, $24,25(OH)_2D_3$, and 3-epi-$25(OH)D_3$ concentrations at 36 months by the treatment group. **b** Plasma $25(OH)D_3$ AUC from 1 to 36 months by the treatment group. **c** Percentage of children achieving at least 50 and 75 nmol/L of plasma $25(OH)D_3$ at 36 months by the treatment group

infant vitamin D policy recommendation; however, larger studies with more ethnically diverse groups are needed to confirm these results.

Acknowledgments The authors would like to thank Dr. Ali Khamessan, PhD Europharm International Canada Inc., for the design of the study product. We thank McGill University graduate student Sade Hayes RD, MSc for the help with study measurements and analysis of the dietary data. Finally, we would like to thank all the families who agreed to participate in the original and current follow-up study and the Mary Emily Clinical Nutrition Research Unit of the School of Dietetics and Human Nutrition.

Research funding was provided by the Canadian Institutes of Health Research and Nutricia Research Foundation, Europharm International Canada Inc. provided in-kind support of supplements. The sponsors were not involved in the design, conduct of the study, collection, management, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript. Dr. Hope A. Weiler, RD PhD is a Canada Research Chair with infrastructure funding from the Canadian Foundation for Innovation. Drs. Sina Gallo and Hope Weiler had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Compliance with ethical standards McGill University and George Mason University institutional review boards provided ethical approval for the study.

Conflicts of interest Sina Gallo, Tom Hazell, Catherine A. Vanstone, Sherry Agellon, Glenville Jones, Mary L'Abbé, and Celia Rodd declare that they have no conflict of interest. Hope Weiler is a Canada Research Chair with infrastructure funding from the Canadian Foundation for Innovation.

Sources of support Canadian Institutes of Health Research and the Nutricia Research Foundation for research funding, and Europharm International Canada Inc. for the in-kind support of supplements.

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