Vitamin D status and functional health outcomes in children aged 2–8 y: a 6-mo vitamin D randomized controlled trial

Neil R Brett,[1] Colleen A Parks,[1] Paula Lavery,[1] Sherry Agellon,[1] Catherine A Vanstone,[1] Martin Kaufmann,[2] Glenville Jones,[2] Jonathon L Maguire,[3] Frank Rauch,[4,5] and Hope A Weiler[1]

[1]School of Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada; [2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada; [3]Applied Health Research Centre, Li Ka Shing Knowledge Institute of St. Michael's Hospital, Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada; [4]Shriners Hospital for Children, Montreal, Quebec, Canada; and [5]Department of Pediatrics, McGill University, Montreal, Quebec, Canada

ABSTRACT

Background: Most Canadian children do not meet the recommended dietary intake for vitamin D.

Objectives: The aims were to test how much vitamin D from food is needed to maintain a healthy serum 25-hydroxyvitamin D_3 [25(OH)D_3] status from fall to spring in young children and to examine musculoskeletal outcomes.

Design: Healthy children aged 2–8 y ($n = 51$) living in Montreal, Canada, were randomly assigned to 1 of 2 dietary vitamin D groups (control or intervention to reach 400 IU/d by using vitamin D–fortified foods) for 6 mo, starting October 2014. At baseline and at 3 and 6 mo, anthropometric characteristics, vitamin D metabolites (liquid chromatography–tandem mass spectrometry), and bone biomarkers (IDS-iSYS, Immunodiagnositc Systems; Liaison; Diasorin) were measured and physical activity and food intakes surveyed. At baseline and at 6 mo, bone outcomes and body composition (dual-energy X-ray absorptiometry) were measured. Cross-sectional images of distal tibia geometry and muscle density were conducted with the use of peripheral quantitative computed tomography scans at 6 mo.

Results: At baseline, participants were aged 5.2 ± 1.9 (mean ± SD) y and had a body mass index z score of 0.65 ± 0.12; 53% of participants were boys. There were no differences between groups in baseline serum 25(OH)D_3 (66.4 ± 13.6 nmol/L) or vitamin D intake (225 ± 74 IU/d). Median (IQR) compliance was 96% (89–99%) for yogurt and 84% (71–97%) for cheese. At 3 mo, serum 25(OH)D_3 was higher in the intervention group ($P < 0.05$) but was not different between groups by 6 mo. Although lean mass accretion was higher in the intervention group ($P < 0.05$), no differences in muscle density or bone outcomes were observed.

Conclusions: The consumption of 400 IU vitamin D/d from fall to spring did not maintain serum 25(OH)D_3 concentration or improve bone outcomes. Further work with lean mass accretion as the primary outcome is needed to confirm if vitamin D enhances lean accretion in healthy young children. This trial was registered at www.clinicaltrials.gov as NCT02387892. *Am J Clin Nutr* 2018;107:355–364.

Keywords: 25-hydroxyvitamin D, randomized controlled trial, children, DXA, muscle, bone

INTRODUCTION

Vitamin D is important for bone growth and development in children (1) and cannot be synthesized from exposure of skin to sunlight at latitudes ≥40°N year-round because solar UV-B radiation is limited in winter months (2). Clinically, vitamin D status is assessed by using serum 25-hydroxyvitamin D [25(OH)D] concentration, a composite reflection of total intake and synthesis. The Institute of Medicine (IOM) recommendations for vitamin D intake were set to meet the needs of the general population in the absence of UV-B exposure. The Estimated Average Requirement (EAR; 400 IU/d) and the Recommended Dietary Allowance (RDA; 600 IU/d) align with serum 25(OH)D concentrations of 40 and 50 nmol/L, respectively (3). Given these recommendations, Canadian children, with an average intake of 244 IU vitamin D/d, appear to be at risk of not meeting population targets for vitamin D status (4).

The IOM vitamin D recommendations for young children were based on results from supplementation trials in adults and older children (3). Since that time, vitamin D interventions in young

Supported by funding from Dairy Farmers of Canada, the Canada Foundation for Innovation and Canada Research Chairs programs, and in-kind support from Agropur and Ultima Foods for the study products. The funding agencies had no role in the design or conduct of the trial. The liquid chromatography–tandem mass spectrometry instrument was provided and maintained by the Waters Corporation (Milford, Massachusetts) as part of a Queen's University/Waters Collaborative Agreement.

Address correspondence to HAW (e-mail: hope.weiler@mcgill.ca).

Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; CTx, C-terminus telopeptide; DXA, dual-energy X-ray absorptiometry; EAR, Estimated Average Requirement; IOM, Institute of Medicine; ITA, individual typological angle; pQCT, peripheral quantitative computed tomography; PTH, parathyroid hormone; P1NP, procollagen type 1 N-terminal propeptide; RDA, Recommended Dietary Allowance; 3-epi-25(OH)D_3, C3 epimer of 25-hydroxyvitamin D^3 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxyvitamin D_3; 24,25$(OH)_2D_3$, 24,25-dihydroxyvitamin.

Received May 17, 2017. Accepted for publication December 7, 2017.

First published online March 16, 2018; doi: https://doi.org/10.1093/ajcn/nqx062.

children have been trialed during the winter months (5–8), and results from 3 of these interventions (5, 7, 8) suggest that the vitamin D EAR may be overestimated for young children. Furthermore, only 1 study (8) had mean 25(OH)D concentrations equivalent to those observed in national surveillance studies conducted in Canada and the United States (children aged 3–11 y: 64–74 nmol/L) (9, 10). Thus, it remains questionable how much vitamin D is required to maintain healthy vitamin D status during seasonal UV-B–void periods. In addition, although there is a well-known positive impact of vitamin D on bone health in adults (3), trial data in young children are limited (11, 12) and it is possible that vitamin D interventions may only affect bone outcomes when baseline 25(OH)D concentrations are <35 nmol/L (13). Data from children in Montreal (aged 2–5 y) showed that vitamin D status ≥75 nmol/L was positively related to bone mineral density (BMD) of the whole body and radius (14) and was also related to a leaner body phenotype (15). This agrees with a 1-y trial in vitamin D–deficient prepubertal girls, in whom lean mass accretion was greater in those receiving a vitamin D supplement (16). Thus, the primary objective was to test how much vitamin D intake from food is required to maintain healthy vitamin D status from the beginning of the UV-B–void period (end of October) to the end of the winter period (March). It was hypothesized that intakes of 400 IU/d, but not 200 IU/d, would support serum 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations of ≥50 nmol/L. The secondary objective was to explore the effects on lean mass, bone mineral accrual, bone geometry, and biomarkers of bone mass and mineral metabolism. Lean mass was not originally specified as a secondary outcome; however, it was added as an outcome due to recent work in Montreal children, which showed a relation of vitamin D status to lean mass (15).

METHODS

Study design

This was a 6-mo, double-blind, randomized controlled trial in Montreal, Canada, following the CONSORT (Consolidated Standards of Reporting Trials) guidelines, with baseline assessments in October 2014. Children were randomly allocated by family in a 1:1 design and stratified by families with only young (aged 2–4 y) or only school-age (aged 5–8 y) children or families with both ages of children to double-blinded groups (**Figure 1**) by using random-numbers tables. Group codes were randomly assigned by a member of the research team with no direct contact with the participants. In addition to their regular food intake, the control group was instructed to consume 33 g cheddar cheese/d or two 93-mL drinkable yogurts/d, neither with added vitamin D (expected vitamin D intake: 140–195 IU/d). To reach the 400 IU/d, the intervention group consumed the same yogurt and cheese products, except with added vitamin D_3. The cheese contained 300 IU vitamin D_3/33 g and yogurt beverages contained 150 IU/93 mL. Children could consume the products at any time during the day so that the products would be incorporated into their normal eating habits. The products were provided precoded by the companies, with codes only disclosed after all of the data were analyzed. The vitamin D content of each product was independently verified to be within ±8% (Maxxam Analytique, Inc.) for the yogurt and within ±5% (O'Neal Scientific Services, Inc.) for the cheese. Families were instructed to otherwise follow their normal lifestyle. Children were seen at baseline and at 3 and 6 mo, at which time anthropometric measures were taken, fasting blood samples were obtained, and surveys were completed on demographic characteristics, illnesses, sun exposure, physical activity, and dietary intake. At baseline and at 6 mo, body composition and bone geometry measures were taken.

Subjects

Children were recruited from daycare centers from August to October 2014. Inclusion criteria were as follows: 2–8 y of age, consuming milk products regularly, within ±2 BMI *z* scores from 0 for sex and age based on WHO growth charts (17) or body fat percentage within normal ranges (18), and not taking supplements containing vitamin D. Exclusion criteria were chronic diseases or medications known to affect vitamin D, known anemia, small size at birth, or preterm birth at <37 wk of gestation.

Assessments

Blood sampling, vitamin D status, and bone biomarkers

Fasting venipuncture samples were taken between 0700 and 1100 to control for diurnal variation. Immediately thereafter, ionized calcium in whole blood (0.1 mL) was measured as a safety assessment by using a blood gas unit (ABL80 FLEX; Radiometer Medical A/S), which had CVs of ≤5%. Two milliliters of whole blood was separated to obtain serum for measurement of 25(OH)D, parathyroid hormone (PTH), osteocalcin, C-terminus telopeptide (CTx), and procollagen type 1 N-terminal propeptide (P1NP).

Samples were prepared for 25(OH)D assessment as previously described (19). Serum 25(OH)D_3, 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$], and C3 epimer of 25(OH)D_3 [3-epi-25(OH)D_3] were quantified by using ultra-HPLC tandem mass spectrometry (Acquity UPLC with Xevo TQ-S mass spectrometer; Waters) at Queen's University, Canada (19). Lower limits of quantification for metabolites were 0.25–0.75 nmol/L. National Institute for Standards and Technology 25(OH)D standards 972a levels 1 and 4 had inter- and intra-assay CVs <5% and an accuracy ≥95%. Intact 1–84 PTH and osteocalcin were measured by using chemiluminescent immunoassays on an autoanalyzer (Liaison; Diasorin). Sensitivity was 2.36 pg/mL for PTH and 3.0 ng/mL for osteocalcin. Controls for PTH and osteocalcin had inter- and intra-assay CVs <7% and an accuracy ≥95%. CTx (45 μL) and P1NP (20 μL diluted 10×) were measured by using chemiluminescent immunoassays at Shriners Hospital for Children (Montreal, Canada) with an IDS-iSYS autoanalyzer (Immunodiagnostic Systems). The CTx assay had a sensitivity of 0.033 ng/mL (range: 0.033–6.000 ng/mL) and P1NP a quantification limit <1.0 ng/mL (dynamic range: 2–230 ng/mL).

Dietary assessment and compliance

A validated 13-item, semiquantitative, 30-d food-frequency questionnaire was used to estimate vitamin D and calcium intakes (20). A 24-h food intake assessment, documented the day before sampling, was used to assess macronutrient and energy intakes.

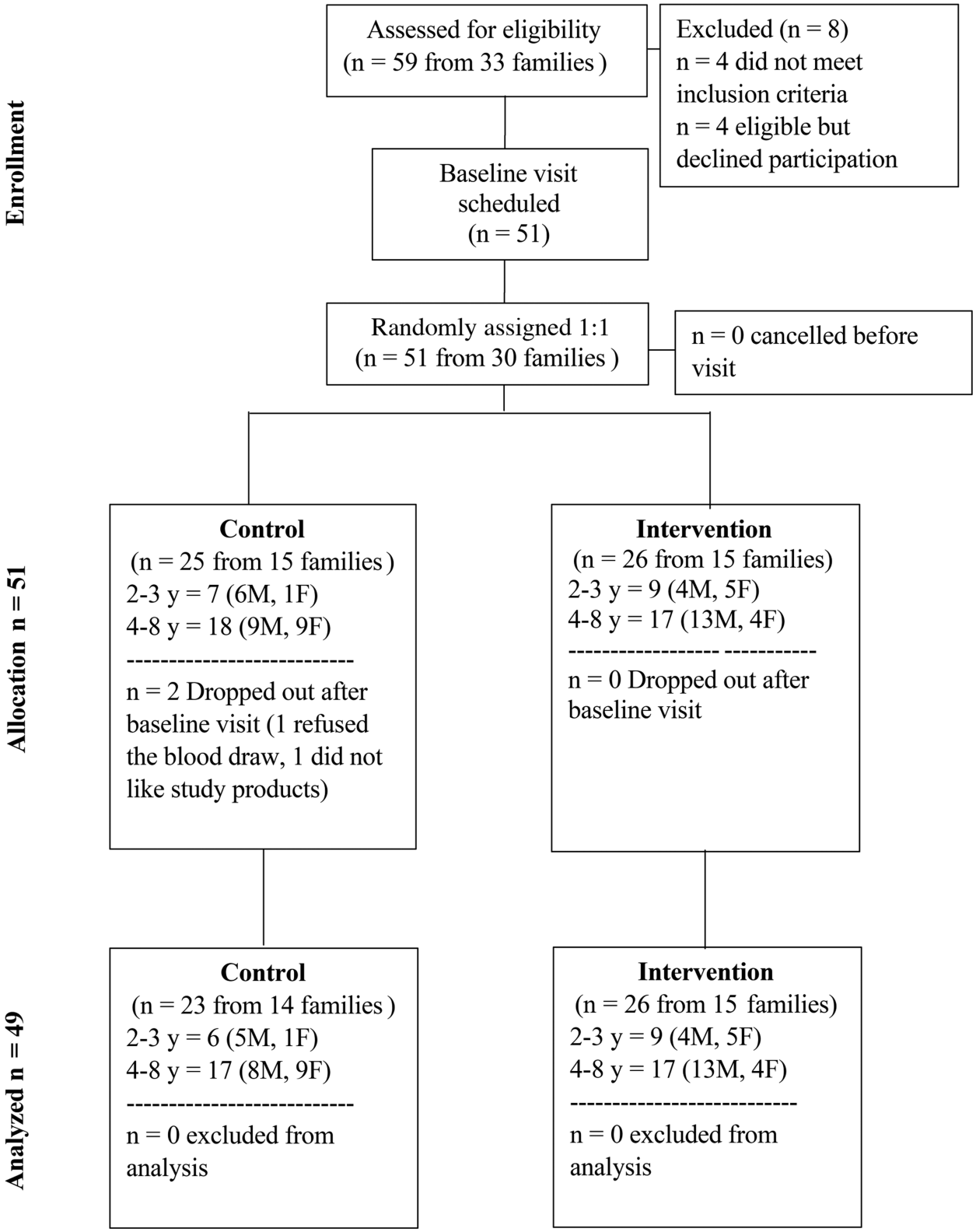

FIGURE 1 CONSORT diagram. CONSORT, Consolidated Standards of Reporting Trials.

Although 3 d of 24-h intake assessments may be sufficient to measure energy and macronutrient intakes, to represent usual intakes of calcium and vitamin D, a longer time period of assessment is needed (21). Thus, the 30-d food-frequency questionnaire was used to measure the intakes of these micronutrients. Nutritionist Pro (Axxya Systems LLC) and the Canadian Nutrient File version 2010b were used to generate nutrient intakes. Parents used daily calendar check sheets, collected and verified every 3 mo, to record compliance. Compliance data were lacking for only one child who finished the trial.

Demographic characteristics, physical activity, skin pigmentation, and UV-B

At baseline, self-reported sociodemographic variables were surveyed. At all visits, data were collected on parent-reported physical activity for weekends and weekdays by using the validated Habitual Activity Estimation Scale questionnaire (22), sun exposure during the previous 30 d, frequency of sunscreen use, and hours spent in direct sunlight per day on the basis of the Canadian Health Measures Survey (23). Skin type was determined by using a spectrophotometer to measure individual typological angle (ITA; CM-700d/600d; Konica Minolta) and UV-B exposure was qualitatively determined as previously described (7).

Anthropometric measurements

Methods for measuring height, weight, and BMI *z* scores were previously described (11). The International Society for Clinical Densitometry states that whole body and lumbar spine are the preferred dual-energy X-ray absorptiometry (DXA) measurement sites (24). In addition, forearm scans were performed because the forearm is the most common fracture site in children (25). For bone outcomes, DXA measures of whole body, lumbar vertebrae 1–4 (anterior-posterior), and distal forearm (nondominant) were made. Ultra-distal forearm data were reported because this region measures new bone close to the growth plate. Whole-body scans were used to assess lean mass. All of the scans were performed by using a Hologic 4500A (APEX software, version 13.3:3) fan-beam clinical densitometer at baseline and at 6 mo. Daily quality-control measurements were obtained by using a lumbar spine bone phantom (Hologic) with an accuracy of ±1.5% of the mean and CVs of 0.396%, 0.524%, 0.363% for BMD, bone mineral content (BMC), and area, respectively. As per the manufacturer's recommendations, global SDs for the low-air and high-air measures of radiographic uniformity were always <2.0.

Peripheral quantitative computed tomography (pQCT; XCT-2000; Stratec) scans of the nondominant tibia were performed by X-ray technicians at 6 mo to evaluate 3-dimensional bone geometry and muscle variables. There are no standard pQCT methods for children (26), so our methods were based on those used when producing normative data in children (27). Length of the tibia was measured between the superior margin of the medial condyle and the medial malleolus. A scout scan was performed to visualize the distal growth plate and the reference line placed at the most proximal line of the growth plate. The 4% and 66% sites, measured proximally from the distal end of the tibia, were each scanned with a single 2-mm slice, a voxel size of 0.4 mm^2, and speed of 30 mm/s.

Ethics

This study was approved by the McGill University Faculty of Medicine Research Ethics Board in accordance with the Tri-Council policy on ethics and the Declaration of Helsinki (28) and was registered at clinicaltrials.gov (NCT02387892). Temporary Marketing Authorization letters were obtained from Health Canada for the trial products (TM-14-0112 and TM-14-0113).

Statistical analyses

Following CONSORT guidelines, we based our sample size calculation on the primary outcome of 25(OH)D_3 and thus did not perform a priori power calculations for secondary outcomes. We aimed to recruit 25 children/group, with an expected group difference of 20 nmol/L and an SD of 16 nmol/L and accounting for a 5–10% drop-out rate (11). Intent-to-treat analyses were conducted by using SAS (version 9.3; SAS Institute). All data entry was double audited and tested for normality by using the Kolmogorov-Smirnov test and homogeneity of variance by using the Bartlett test. A P value <0.05 was categorized as significant, after adjustment for multiple comparisons where applicable. A mixed-model ANOVA was used to analyze continuous data, accounting for fixed effects (group, sex, and age) and random effects (e.g., within family, demographic characteristics, body composition), with post hoc testing where necessary by using Bonferroni correction. Non-normal data were log-transformed where applicable [e.g., 25(OH)D_3]. The drop-out rate was <5% during the study period, so data imputation approaches were not sought. Fisher's exact testing was used for differences in proportions. Log-log regression analysis was used to normalize fat mass and lean mass indexes (29), because fat mass and lean mass have different relations with height in children than does weight.

RESULTS

Demographic characteristics

No differences in baseline characteristics (**Table 1**) were observed between allocation groups. Physical activity was not different between groups, by using Habitual Activity Estimation Scale questionnaire activity categories, in which 91% and 82% of control and intervention groups, respectively, were very active for ≥60 min. Forty-nine (96.1%) children completed the study (Figure 1) over (mean ± SD) 25.3 ± 0.6 wk. There were no differences in height (control: 112.5 ± 15.0 cm; intervention: 111.0 ± 13.5 cm), weight (control: 22.3 ± 7.1 kg; intervention: 20.3 ± 5.1 kg), height velocity (control: 0.5 ± 0.2 cm/mo; intervention: 0.5 ± 0.1 cm/mo), or weight velocity (control: 0.3 ± 0.1 kg/mo; intervention: 0.2 ± 0.2 kg/mo) between groups (**Table 2**).

Dietary characteristics and sun exposure

Median (IQR) compliance for the study yogurt and cheese was 96% (89–99%) and 84% (71–97%), with no differences between groups. Mean milk and alternatives intake (baseline, 3 mo, and

TABLE 1
Baseline characteristics of participants[1]

	Control	Intervention	P[2]
n	25	26	
Age, y	5.4 ± 2.0[3]	5.0 ± 1.8	0.752
Range	1.9–8.6	2.0–8.4	
Male sex, *n* (%)	15 (56)	12 (50)	0.300
White ethnicity,[4] *n* (%)	13 (52)	18 (69)	0.264
Maternal education, college or higher, *n* (%)	20 (80)	18 (69)	0.172
Family income, *n* (%)			0.065
>$65,000[5]	18 (72)	12 (50)	
Not disclosed	0	1 (4)	
z Score			
Weight	0.75 ± 0.87	0.64 ± 1.09	0.874
Height	0.31 ± 0.90	0.44 ± 1.10	0.413
BMI	0.81 ± 0.88	0.55 ± 0.98	0.275
Serum 25(OH)D_3, *n* (%)			0.313
<30 nmol/L	0	0	
30–39.9 nmol/L	0	0	
40–49.9 nmol/L	2 (8)	2 (8)	
50–124.9 nmol/L	23 (92)	24 (92)	
≥125 nmol/L	0	0	

[1]There were no differences between groups at baseline. 25(OH)D_3, 25-hydroxyvitamin D_3.
[2]Derived by testing for differences between groups with the use of a mixed-model ANOVA or Fisher's exact test.
[3]Unadjusted mean ± SD (all such values).
[4]Nonwhite = Hispanic, black, or Asian.
[5]Canadian dollars.

6 mo: 2.8 ± 0.7, 2.7 ± 1.0, and 3.2 ± 1.4 servings/d, respectively) exceeded Canada's Food Guide recommendations (2 servings/d). At baseline, vitamin D intakes were not different between groups and no children had intakes ≥400 IU/d (**Table 3**). The vitamin D intake of the control group did not change throughout the study, whereas at 3 and 6 mo, the intervention group significantly differed from control (Table 3). Calcium intake was not different between groups at any time point (Table 3). Baseline median (IQR) energy intake [control: 1670 kcal/d (1416–2020 kcal/d); intervention: 1420 kcal/d (1302–1682 kcal/d)] and protein intake [control: 77 g/d (66–84 g/d); intervention: 69 g/d (45–76 g/d)] did not differ between groups or change at any time point.

Sixty-seven percent (34 of 51) of the children had Fitzpatrick skin types I, II, or III and 33% (17 of 51) had skin types IV, V, or VI. Six percent (3 of 51) of children traveled to southern latitudes during the study period and none of them presented with significant tanning of skin as measured by changes in ITA at the forearm, forehead, and lower leg [ΔITA (average of 3 sites): 5.6° ± 4.6°].

TABLE 2
Anthropometric measurements and growth of children over the 6-mo study[1]

Group	Height, cm	Height velocity, cm/mo	Weight, kg	Weight velocity, kg/mo
Baseline				
Control	112.5 ± 15.0	N/A	22.1 ± 7.3	N/A
Intervention	111.0 ± 13.5	N/A	20.3 ± 5.1	N/A
6 mo				
Control	115.2 ± 14.4	0.5 ± 0.2	22.9 ± 7.2	0.3 ± 0.1
Intervention	114.2 ± 14.2	0.5 ± 0.1	21.8 ± 6.1	0.2 ± 0.2

[1]Values are means ± SDs. There were no significant differences between groups with the use of a mixed-model ANOVA, adjusted for age, sex, ethnicity, family cluster, and length of study. N/A, not applicable.

TABLE 3
Intakes of calcium, vitamin D, energy, and serum 25(OH)D_3 concentration across the 6-mo study[1]

	Control	Intervention
Calcium[2]		
Baseline, mg/d	909 ± 223	946 ± 351
Meeting EAR, %	64[a,b]	73[a,b]
3 mo, mg/d	842 ± 262	869 ± 363
Meeting EAR, %	48[a]	62[a,b]
6 mo, mg/d	1066 ± 360	1034 ± 391
Meeting EAR, %	87[b]	77[b]
Vitamin D[2]		
Baseline, IU/d	202 ± 76[a]	248 ± 73[a]
Meeting 400 IU/d, %	0[a]	0[a]
3 mo, IU/d	239 ± 117[a]	466 ± 95[b]
Meeting 400 IU/d, %	12[a]	81[b]
6 mo, IU/d	241 ± 124[a]	486 ± 90[b]
Meeting 400 IU/d, %	12[a]	81[b]
25(OH)D_3		
Baseline, nmol/L	67.5 ± 15.1[a]	65.3 ± 12.2[a]
Meeting 50 nmol/L, %	92[a]	92[a]
3 mo, nmol/L	58.3 ± 15.3[b]	64.7 ± 12.2[a]
Meeting 50 nmol/L, %	67[b]	88[a,b]
6 mo, nmol/L	56.6 ± 13.9[b]	58.4 ± 8.7[b]
Meeting 50 nmol/L, %	70[a,b]	85[a,b]
Energy,[3] kcal/d		
Baseline	1670 (1416–2020)	1420 (1302–1682)
3 mo	1527 (1273–1821)	1540 (1345–1799)
6 mo	1583 (1160–1878)	1503 (1362–1647)

[1]Values are unadjusted means ± SDs or medians (IQRs). Control group—baseline: $n = 25$; 3 mo: $n = 23$; 6 mo: $n = 23$. Intervention group—baseline: $n = 26$; 3 mo: $n = 26$; 6 mo: $n = 26$. Different superscript letters denote significant differences between groups and over time ($P = 0.001$–0.042). Fisher's exact test was used to test for between-group differences in the proportion meeting recommendations. A mixed-model ANOVA with Bonferroni correction, adjusted for age, sex, ethnicity, BMI *z* score, family cluster, length of study, and baseline serum 25(OH)D_3, was used to test for between-group differences in continuous variables. EAR, Estimated Average Requirement; 25(OH)D_3, 25-hydroxyvitamin D_3.
[2]From a validated 30-d food-frequency questionnaire.
[3]From 24-h intake assessments.

Biochemical assessments

At baseline, serum 25(OH)D_3 ranged between 40 and 125 nmol/L (Table 1), with mean concentrations of 66.4 ± 13.6 nmol/L overall. With regard to our primary outcome, the control group showed a decrease in 25(OH)D_3 ($P = 0.001$) from 0 to 3 mo, and the intervention group showed a decrease ($P = 0.001$) from 3 to 6 mo (**Figure 2**A). This resulted in serum 25(OH)D_3 being lower in the control group than in the intervention group at 3 mo ($P = 0.001$) but not at 6 mo. Compliance did not significantly influence serum 25(OH)D_3, and baseline 25(OH)D_3 was not predictive of the 6-mo change in 25(OH)D_3. No child at any time point had 25(OH)D_3 concentrations >125 nmol/L, and only 1 child (4.3%) in the control group had 25(OH)D_3 <40 nmol/L (39.4 nmol/L) at 6 mo. The

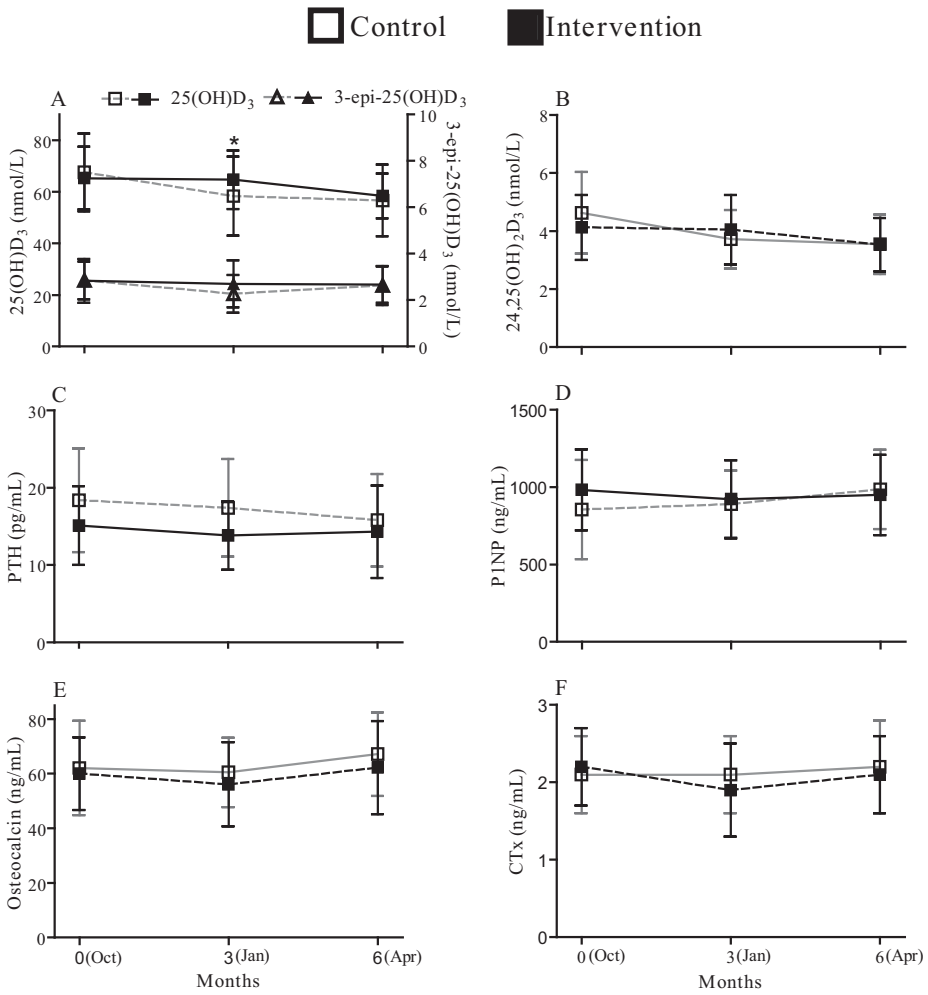

FIGURE 2 Serum 25(OH)D_3 and 3-epi-25(OH)D_3 (A), 24,25$(OH)_2D_3$ (B), PTH (C), P1NP (D), osteocalcin (E), and CTx (F) concentrations at baseline and at 3 and 6 mo. Values are means ± SDs. *Different between groups at that time point ($P = 0.001$). For 25(OH)D_3 and 24,25$(OH)_2D_3$, both groups significantly decreased ($P = 0.001$) from baseline to 6 mo. The group by time interaction for 25(OH)D_3 was $P = 0.004$. Analyses used a mixed-model ANOVA with Bonferroni correction adjusted for age, sex, ethnicity, BMI *z* score, family cluster, length of study, and baseline serum 25(OH)D_3. CTx, C-terminal telopeptide; PTH, parathyroid hormone; P1NP, procollagen type 1 N-terminal propeptide; 3-epi-25(OH)D_3, C3 epimer of 25-hydroxyvitamin D_3; 25(OH)D_3, 25-hydroxyvitamin D_3; 24,25$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3.

proportion of children with 25(OH)D_3 ≥75 nmol/L was not different between groups at any time [baseline—control: 7 of 25 (28%); intervention: 6 of 26 (23%); 3 mo—control: 4 of 24 (16%); intervention: 6 of 26 (23%); 6 mo—control: 1 of 23 (4%); intervention: 2 of 26 (8%)] but significantly decreased only in the control group over 6 mo ($P = 0.049$). Similarly, the proportion of children with 25(OH)D_3 <40 nmol/L or ≥50 nmol/L was not different between groups at any time but significantly decreased in the control group at 3 mo (Table 3). Serum 24,25$(OH)_2D_3$ decreased (−1.0 ± 1.3 nmol/L; $P = 0.001$) over time (Figure 2B). The 25(OH)D_3-to-24,25$(OH)_2D_3$ ratio (range of ratio: 10.1–25.0) and 3-epi-25(OH)D_3 did not change over time or differ between groups (Figure 2A).

Average ionized calcium (1.30 ± 0.04 mmol/L) was within normal limits (1.15–1.38 mmol/L) and did not vary by time or treatment group. PTH, P1NP, osteocalcin, and CTx concentrations did not differ over time or between groups (Figure 2C–F). Concentrations of biomarkers did not vary on the basis of the time of blood draw (0700–1100).

Body-composition assessments

Fat mass index (control: 4.85 ± 1.31 kg/$m^{1.9}$; intervention: 4.56 ± 1.43 kg/$m^{1.9}$) as well as whole-body fat mass and appendicular regions as a total and legs alone (**Table 4**) were not different between groups or over time. Lean mass index (control: 11.29 ± 1.07 kg/$m^{2.5}$; intervention: 10.85 ± 0.76 kg/$m^{2.5}$) did not differ between groups or over time. However, the mean (95% CI) percentage increase in whole-body [control: 5.9% (4.4–7.4%); intervention: 8.4% (6.3–10.5%)], appendicular [control: 7.7% (5.7–11.3%); intervention: 13.3% (8.9–17.1%)], and legs-alone [control: 8.9% (4.1–12.7%); intervention: 15.7% (11.7–19.6%)] lean mass was significantly greater over 6 mo in the intervention group (whole body, $P = 0.038$; appendicular, $P = 0.045$; legs, $P = 0.025$). In addition, the 6-mo absolute changes in lean mass for the whole body (control: 0.31 ± 0.67 kg; intervention: 0.98 ± 0.59 kg; $P = 0.038$) and the appendicular skeleton (control: 0.35 ± 0.71 kg; intervention: 0.67 ± 0.58 kg; $P = 0.034$) were significantly greater in the intervention group (Table 4). The

TABLE 4
Bone and body-composition variables from DXA scans in children aged 2–8 y across the 6-mo study period[1]

	Control		Intervention		P[2]		
	Baseline	6 mo	Baseline	6 mo	Time	Group	Group 6-mo absolute change
Whole-body							
BMD, g/cm^2	0.696 ± 0.093	0.711 ± 0.093	0.691 ± 0.118	0.704 ± 0.119	0.73	0.15	0.98
BMC, g	805.78 ± 202.83	865.73 ± 223.90	731.13 ± 185.93	769.03 ± 198.55	0.89	0.38	0.09
Lumbar spine							
BMD, g/cm^2	0.519 ± 0.072	0.523 ± 0.076	0.502 ± 0.073	0.513 ± 0.073	0.55	0.04	0.62
BMC, g	16.68 ± 4.48	18.40 ± 5.29	15.18 ± 4.18	16.11 ± 3.79	0.56	0.11	0.10
U-D forearm							
BMD, g/cm^2	0.267 ± 0.06	0.288 ± 0.07	0.238 ± 0.04	0.245 ± 0.03	0.42	0.004	0.12
BMC, g	0.93 ± 0.3	1.03 ± 0.3	0.77 ± 0.2	0.82 ± 0.2	0.45	0.01	0.08
Lean mass, kg							
Whole-body	15.33 ± 4.89	15.64 ± 4.89	14.18 ± 4.11	15.16 ± 4.25	0.77	0.27	0.038
Appendicular	6.76 ± 2.96	7.11 ± 2.75	5.92 ± 2.26	6.59 ± 2.33	0.98	0.79	0.034
Legs	4.56 ± 1.96	4.86 ± 2.05	3.86 ± 1.65	4.37 ± 1.72	0.89	0.94	0.10
Fat mass, kg							
Whole-body	6.10 ± 2.37	6.23 ± 2.56	5.34 ± 2.10	5.48 ± 2.20	0.81	0.73	0.51
Appendicular	3.44 ± 1.46	3.75 ± 1.76	2.81 ± 1.09	2.89 ± 1.19	0.99	0.34	0.58
Legs	2.70 ± 1.10	2.79 ± 1.20	2.34 ± 0.86	2.35 ± 0.95	0.64	0.50	0.61

[1] Values are unadjusted means ± SDs or means (95% CIs). Control—baseline: $n = 24$; 6 mo: $n = 22$ (1 child without scan at each time point due to lack of cooperation). Intervention—baseline: $n = 26$; 6 mo: $n = 25$ (1 child without scan at 6 mo due to lack of cooperation). BMC, bone mineral content; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; U-D, ultra-distal nondominant; 25(OH)D_3, 25-hydroxyvitamin D.

[2] P values are shown for differences between baseline and 6 mo, differences between groups, and differences between groups for the 6-mo absolute change in each variable. A mixed-model ANOVA with Bonferroni correction was used and adjusted for age, sex, ethnicity, height velocity, family cluster, length of study, and baseline serum 25(OH)D_3.

absolute change in lean mass of the legs was not different between groups (control: 0.30 ± 0.40 kg; intervention: 0.51 ± 0.23 kg; $P = 0.10$). Lean mass outcomes were not related to physical activity. Although there were between-group differences in lean mass accretion, lower leg muscle density and cross-sectional area were not different between groups at 6 mo (**Table 5**).

Bone assessments

Bone outcomes did not differ between groups. Specifically, BMD z scores (whole-body z score—control: 1.46 ± 1.36; intervention: 0.90 ± 0.91; lumbar spine z score—control: 0.54 ± 0.90; intervention: 0.18 ± 0.85) were not different between groups and did not change over the 6 mo. Radius (33%) BMD z scores (range: −1.2 to 1.9) were not available for all ages. The groups did not significantly differ in BMC accretion rates for whole body, lumbar spine and ultra-distal forearm over time (Table 4). None of the pQCT-derived bone outcomes (Table 5) were different between groups.

TABLE 5
Bone and muscle variables from peripheral quantitative computed tomography scans of the lower leg in children aged 2–8 y after 6 mo of study[1]

	Control	Intervention
Tibia geometry		
4% Trabecular density, mg/cm^3	179.3 ± 32.6	187.1 ± 25.2
4% Trabecular CSA, mm^2	189.5 ± 35.0	179.8 ± 50.2
66% Cortical density, mg/cm^3	986.8 ± 68.1	994.0 ± 47.3
66% Cortical CSA, mm^2	114.7 ± 39.4	116.5 ± 42.5
66% Cortical thickness, mm	0.47 ± 0.23	0.41 ± 0.13
Muscle variables		
66% Muscle density, mg/cm^3	56.4 ± 15.9	51.6 ± 11.1
66% Muscle CSA, mm^2	2696.1 ± 758.8	2465.0 ± 550.0

[1] Values are unadjusted means ± SDs. Control: $n = 22$; intervention: $n = 25$ (1 child without scans for each group due to lack of cooperation). There were no differences between groups ($P > 0.05$) for outcomes with the use of a mixed-model ANOVA, adjusted for age, sex, ethnicity, and family cluster. CSA, cross-sectional area.

DISCUSSION

The IOM recommendations for vitamin D were set on the basis of evidence from adolescents and adults (3), highlighting the need for randomized controlled trials in young children. To address this gap, the present study suggests that 400 IU vitamin D/d maintains serum 25(OH)D ≥40 nmol/L in 100%, ≥50 nmol/L in 85%, and ≥75 nmol/L in 8% of young children during UV-B–void periods in Canada. Despite interim benefits at 3 mo, the intervention did not maintain vitamin D status over 6 mo compared with the control group. It is possible that if we had used greater amounts of vitamin D that the intervention group would have had a higher status; however, that was not suitable for a fortified food–based trial because such foods would not realistically be approved for the Canadian food market (4). Within the secondary outcomes, greater lean mass accretion was observed in the intervention group and may imply that meeting 400 IU/d for vitamin D could be beneficial for physical development beyond bone health. Because this finding is hypothesis-generating, further work is needed to confirm if vitamin D has an effect on lean mass in healthy young children.

The intervention in this study was designed to achieve dietary intakes of 400 IU vitamin D/d, consistent with the EAR and below

the RDA (600 IU/d). Although the intervention failed to support the hypothesized 25(OH)D_3 concentration of 75 nmol/L, it did maintain 25(OH)D_3 at >40 nmol/L in all participants with 85–88% of values ≥50 nmol/L, which is more similar to that anticipated when achieving the RDA. The baseline serum 25(OH)D_3 of ∼70 nmol/L in our study is similar to national surveillance data (3–5 y: 74 nmol/L; 6–11 y: 67 nmol/L) (9). In contrast, trials in children aged 4–8 y in Denmark (5) and aged 8–14 y in Pittsburgh (6) suggest that 780 and 1500 IU/d, respectively, are needed for 97.5% of children in winter to maintain 25(OH)D ≥50 nmol/L. Both trials had lower baseline 25(OH)D (Danish children: 56.7 ± 12.3 nmol/L; Pittsburgh children: 50 ± 7.7 nmol/L), which meant that almost half of the children had to first increase their 25(OH)D concentration to 50 nmol/L before maintaining it. Although designed to influence national policy, these studies did not reflect national data [US NHANES 2001–2006 for children aged <11 y—geometric means: 64–69 nmol/L (10); Danish children aged 4–17 y—25(OH)D in the fall: 72.8 nmol/L; IQR: 64.0–88.9 nmol/L (8)]. In addition, the higher intakes needed among the Pittsburgh children may be due to overweight and obese status because the median BMI (kg/m^2; 20.6; IQR: 17.8–23.3) was in the 80–85th percentile (17), although the Danish and Montreal children had mean BMI z scores of 0.08 ± 0.83 and 0.67 ± 0.94, respectively. These points highlight that extrapolation of data from studies in which baseline 25(OH)D or BMI are not reflective of a general population needs careful consideration before applying them to dietary recommendations.

Multiple pediatric trials have shown that adiposity is negatively associated with serum 25(OH)D (30–32). Vitamin D can be absorbed into fat cells instead of being quickly hydroxylated to 25(OH)D because serum 25(OH)D >15 nmol/L is associated with saturation of liver 25-hydroxylase (CYP2R1) (33). It has previously been suggested that adipose tissue may have a negative relation with vitamin D status due to vitamin D sequestration (34). Our data show that fat mass did not significantly affect serum 25(OH)D_3 concentrations observed at 3 or 6 mo. This could be ascribed to the healthy body composition of the participants (by design) and the fact that we adjusted for age, because older children would have higher fat mass than those at the lower end of our age spectrum. However, because 25(OH)D_3 declined over the study and fat mass was relatively stable, it is possible that adipose stores of vitamin D were not sufficient or that needs for tissue expansion were increased, both of which are consistent with a significant decline in serum 24,25$(OH)_2D_3$ after 3 mo and greater lean mass accretion in the intervention group. It has been shown that increased vitamin D intake could be taken up by multiple other body tissues (35), although little is known about vitamin D tissue distribution in children.

Early work on 25(OH)D tissue distribution (36, 37) showed that 25–66% was found in muscle tissue. A 2009 review (33) stated that, in an average woman, ∼20% of 25(OH)D is in muscle tissue (33), suggesting that muscle consumes a significant proportion of 25(OH)D. Calcitriol is implicated in regulating the expression of transcription factors within the myocyte, which is important for muscle development (38), as well as increasing serum concentrations of insulin-like growth factor binding protein 3 (IGFBP3) (39) and activation of calmodulin-dependent kinases (40). Increased IGFBP3 has been suggested to increase the half-life of insulin-like growth factor I, resulting in an increased concentration in circulation and an increase in downstream protein synthesis (39). Calmodulin-dependent kinases were shown to enhance vitamin D receptor–mediated transcription activity and thus may have a synergistic effect with vitamin D on vitamin D receptor–mediated transcription (40). The results of our trial support the hypothesis that a vitamin D intake of 400 IU/d may enhance lean mass accretion in healthy young children. Furthermore, although there was no absolute difference in leg lean mass accretion between groups, the biggest percentage of difference in lean mass accretion between groups was in the legs, similar to results in postmenopausal women (41). With physical activity in our trial not being related to lean mass, it is hypothesized that vitamin D interventions may have a larger effect on fast-twitch muscle fibers (42). Our results also agree with previous trials in Chinese girls (age 15 y) (43) and prepubertal Lebanese girls (aged 10–13 y) (16), which showed significant associations between lean mass and vitamin D intake. In the trial in Lebanese girls, when compared with the control group, supplementing with 200 IU vitamin D/d significantly increased lean mass accretion but not vitamin D status, suggesting that differences in vitamin D intake and not status led to changes in lean mass (16). However, a recent study (15) showed that if serum 25(OH)D was ≥75 nmol/L, vitamin D intakes >400 IU/d did not improve accretion. This underscores the importance of using the serum 25(OH)D concentration measurement when examining the interrelations with lean mass accretion.

The serum 25(OH)D concentration recommended by the IOM for bone health is 50–125 nmol/L (3). All DXA-based BMD z scores were within a healthy range, which agrees with a 2012 meta-analysis (13) showing that vitamin D supplementation in children with serum 25(OH)D >35 nmol/L did not affect hip or lumbar spine BMD. Interestingly, a 1-y trial in Finnish girls (n = 228; mean age: 11.4 ± 0.4 y) showed that vitamin D supplementation elevated total hip BMD without significantly affecting serum 25(OH)D (baseline: 46.3 ± 17.4 nmol/L) (44). This highlights that associations between BMD and vitamin D status need careful examination. With no differences between groups for bone accretion or density outcomes, it is not surprising that in our study we did not find between-group differences for bone biomarkers. Importantly, however, bone health biomarkers can rapidly respond to changes in nutritional status, because P1NP was shown to significantly increase over a time frame as short as 4 wk due to zinc supplementation (45). Thus, if we had seen significant between-group differences in bone accretion in our 6-mo trial, we would have expected significant increases in markers of bone formation.

Significant strengths of our study include a high compliance rate and a 6-mo UV-B–void period with study visits every 3 mo, which provided the ability to track seasonal changes in serum 25(OH)D. By assessing other vitamin D metabolites, we could see whether vitamin D intake related to the conversion of 25(OH)D_3 to 24,25$(OH)_2D_3$. A limitation of our trial was that few children started with 25(OH)D_3 ≥75 nmol/L, which meant that we were not able to test if this status could be maintained or affect functional outcomes. In addition, by focusing on the population target for vitamin D intake (400 IU/d) (3), we were not able to test if higher amounts would better support serum 25(OH)D_3 concentration or other functional outcomes. It is possible that either a longer time frame or a larger sample size would be needed to investigate functional outcomes, especially given the healthy

vitamin D status throughout the study. However, as stated in our methods, DXA has an accuracy of ±1.5% and CVs for BMD and BMC were <0.55%. Thus, the 5–10% changes in BMC (shown in Table 3) and over 6 mo are large enough to be outside the range of instrumental error, meaning that 6 mo was long enough to measure change in BMC. Because pQCT scans were only performed at 6 mo, we were not able to look at changes in bone or muscle outcomes measured by pQCT. In addition, with scans at 4% and 66% sites of the lower leg, we were not able to assess bone outcomes at predominantly cortical sites. Furthermore, we did not look at whether muscle strength of the children was affected by vitamin D intake as has been shown previously (43, 46). Last, our sample of families may be more health conscious or health literate than average Canadians, because the majority of parents in our study were university educated and had family incomes at or above the national average.

In conclusion, 96% of all children maintained 25(OH)D_3 ≥40 nmol/L and 70% of the control group (~200 IU vitamin D/d) maintained 25(OH)D_3 ≥50 nmol/L (set to align with bone health), whereas 85% of the intervention group (~400 IU vitamin D/d) achieved this healthy target. By 6 mo, only 2 children achieved the 75-nmol/L target in the intervention group, because serum 25(OH)D_3 concentrations in both groups declined over the study, which shows that vitamin D stores are utilized during the UV-B–void period but not depleted. The increased vitamin D intake of the intervention group did not lead to improved bone health outcomes, but may explain the higher lean mass accretion in the intervention group than in the control group. Further longer-term studies with lean mass as the primary outcome are needed to confirm this hypothesis-generating finding. These results show a need for future vitamin D food fortification trials to examine the relations between vitamin D intake, serum 25(OH)D, and lean mass outcomes in children. Such trials will further inform dietary recommendations and food fortification policies.

We thank Sandra Dell'Elce for help with procuring blood samples and McGill University graduate student Laura Plante and undergraduate students Kristina Parsons and Kimberley O'Keefe for help with dietary assessment and analysis and study visits. Ms. Plante received financial remuneration for her assistance in dietary assessment. We also thank Pina Napoletano and Donna Baker for their pQCT expertise as well as Sami Abdullah and Elizabeth Parr for their expertise quantifying bone biomarkers P1NP and CTx.

The authors' responsibilities were as follows—HAW, JLM, and FR: designed the research; NRB, CAP, CAV, PL, SA, MK, GJ, and HAW: conducted the research; FR: provided essential materials; NRB and HAW: analyzed the data; NRB, CAV, and HAW: wrote the manuscript; HAW: had primary responsibility for final content; and all authors: revised the manuscript and read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES

1. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009;19(2):73–78.
2. Webb AR, Engelsen O. Ultraviolet exposure scenarios: risks of erythema from recommendations on cutaneous vitamin D synthesis. Adv Exp Med Biol 2014;810:406–22.
3. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. Clarification of DRIs for calcium and vitamin D across age groups. J Am Diet Assoc 2011;111(10):1467.
4. Shakur YA, Lou W, L'Abbe MR. Examining the effects of increased vitamin D fortification on dietary inadequacy in Canada. Can J Public Health 2014;105(2):e127–32.
5. Mortensen C, Damsgaard CT, Hauger H, Ritz C, Lanham-New SA, Smith TJ, Hennessy Á, Dowling K, Cashman KD, Kiely M. Estimation of the dietary requirement for vitamin D in white children aged 4–8 y: a randomized, controlled, dose-response trial. Am J Clin Nutr 2016;104(5):1310–7.
6. Rajakumar K, Moore CG, Yabes J, Olabopo F, Haralam MA, Comer D, Holick MF, Greenspan SL. Estimations of dietary vitamin D requirements in black and white children. Pediatr Res 2016;80(1):14–20.
7. Brett NR, Lavery P, Agellon S, Vanstone CA, Maguire JL, Rauch F, Weiler HA. Dietary vitamin D dose-response in healthy children 2 to 8 y of age: a 12-wk randomized controlled trial using fortified foods. Am J Clin Nutr 2016;103(1):144–52.
8. Madsen KH, Rasmussen LB, Andersen R, Molgaard C, Jakobsen J, Bjerrum PJ, Andersen EW, Mejborn H, Tetens I. Randomized controlled trial of the effects of vitamin D-fortified milk and bread on serum 25-hydroxyvitamin D concentrations in families in Denmark during winter: the VitmaD study. Am J Clin Nutr 2013;98(2):374–82.
9. Janz T, Pearson C. Vitamin D blood levels of Canadians. Statistics Canada; 2013 [Internet]. [cited 2015 Dec 1]. Available from: http://www.statcan.gc.ca/pub/82-624-x/2013001/article/11727-eng.htm .
10. Ganji V, Zhang X, Tangpricha V. Serum 25-hydroxyvitamin D concentrations and prevalence estimates of hypovitaminosis D in the US population based on assay-adjusted data. J Nutr 2012;142(3):498–507.
11. Ekbote V, Khadilkar A, Chiplonkar S, Hanumante N, Khadilkar V, Mughal M. A pilot randomized controlled trial of oral calcium and vitamin D supplementation using fortified laddoos in underprivileged Indian toddlers. Eur J Clin Nutr 2011;65(4):440–6.
12. Hettiarachchi M, Lekamwasam S, Liyanage C. Long term cereal-based nutritional supplementation improved the total spine bone mineral density amongst Sri Lankan preschool children: a randomized controlled study. J Pediatr Endocrinol Metab 2010;23(6):555–63.
13. Winzenberg TM, Powell S, Shaw KA, Jones G. Cochrane Review: vitamin D supplementation for improving bone mineral density in children. Evid-Based Child Health 2012;7(1):294–386.
14. Hazell TJ, Pham TT, Jean-Philippe S, Finch SL, El Hayek J, Vanstone CA, Agellon S, Rodd CJ, Weiler HA. Vitamin D status is associated with bone mineral density and bone mineral content in preschool-aged children. J Clin Densitom 2015;18(1):60–7.
15. Hazell T, Gallo S, Vanstone C, Agellon S, Rodd C, Weiler H. Vitamin D supplementation trial in infancy: body composition effects at 3 years of age in a prospective follow-up study from Montréal. Pediatr Obes 2016;12(1):38–47.
16. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab 2006;91(2):405–12.
17. WHO. Child Growth Standards. 2013 [Internet]. [cited 2013 Dec]. Available from: http://www.who.int/childgrowth/standards/bmi_for_age/en/.
18. Kelly TL, Wilson KE, Heymsfield SB. Dual energy X-ray absorptiometry body composition reference values from NHANES. PLOS One 2009;4(9):e7038.
19. Kaufmann M, Gallagher JC, Peacock M, Schlingmann K-P, Konrad M, DeLuca HF, Sigueiro R, Lopez B, Mourino A, Maestro M. Clinical utility of simultaneous quantitation of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD. J Clin Endocrinol Metab 2014;99(7):2567–74.
20. El Hayek J, Pham TT, Finch S, Hazell TJ, Jean-Philippe S, Vanstone CA, Agellon S, Rodd C, Rauch F, Weiler HA. Vitamin D status in Montreal preschoolers is satisfactory despite low vitamin D intake. J Nutr 2013;143(2):154–60.
21. Bingham S, Gill C, Welch A, Day K, Cassidy A, Khaw K, Sneyd M, Key T, Roe L, Day N. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. Br J Nutr 1994;72(04):619–43.
22. Hay JA, Cairney J. Development of the Habitual Activity Estimation Scale for clinical research: a systematic approach. Pediatr Exerc Sci 2006;18(2):193.
23. Statistics Canada. Canadian Health Measures Survey (cycle 4) Household Questionnaire [Internet]. [cited 2017 Jul]. Available

from: www23.statcan.gc.ca/imdb-bmdi/instrument/5071_Q1_V4-eng.pdf.
24. Baim S, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Lewiecki EM, Silverman S. Official positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. J Clin Densitom 2008;11(1):75–91.
25. Pannu GS, Herman M. Distal radius-ulna fractures in children. Orthop Clin North Am 2015;46(2):235–48.
26. Zemel B, Bass S, Binkley T, Ducher G, Macdonald H, McKay H, Moyer-Mileur L, Shepherd J, Specker B, Ward K. Peripheral quantitative computed tomography in children and adolescents: the 2007 ISCD Pediatric Official Positions. J Clin Densitom 2008;11(1):59–74.
27. Moyer-Mileur LJ, Quick JL, Murray MA. Peripheral quantitative computed tomography of the tibia: pediatric reference values. J Clin Densitom 2008;11(2):283–94.
28. Canadian Institutes of Health Research; Natural Sciences and Engineering Research Council of Canada; Social Sciences and Humanities Research Council. Tri-Council Policy statement: Ethical conduct for research involving humans. 2014 [Internet]. [cited 2016 May 20]. Available from: http://www.pre.ethics.gc.ca/pdf/eng/tcps2-2014/TCPS_2_FINAL_Web.pdf .
29. Wells JC, Cole TJ. Adjustment of fat-free mass and fat mass for height in children aged 8 y. Int J Obes Relat Metab Disord 2002;26(7):947–52.
30. Gilbert-Diamond D, Baylin A, Mora-Plazas M, Marin C, Arsenault JE, Hughes MD, Willett WC, Villamor E. Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study. Am J Clin Nutr 2010;92(6):1446–51.
31. Rajakumar K, Fernstrom JD, Holick MF, Janosky JE, Greenspan SL. Vitamin D status and response to vitamin D3 in obese vs. non-obese African American children. Obesity 2008;16(1):90–95.
32. Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo D-h, Thomas J. A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. J Clin Endocrinol Metab 2010;95(10):4584–91.
33. Heaney RP, Horst RL, Cullen DM, Armas LA. Vitamin D3 distribution and status in the body. J Am Coll Nutr 2009;28(3):252–6.
34. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72(3):690–3.
35. Rosenstreich SJ, Rich C, Volwiler W. Deposition in and release of vitamin D3 from body fat: evidence for a storage site in the rat. J Clin Invest 1971;50(3):679.
36. Mawer EB, Backhouse J, Holman CA, Lumb G, Stanbury S. The distribution and storage of vitamin D and its metabolites in human tissues. Clin Science 1972;43(3):413–31.
37. Cruickshank E, Kodicek E, Armitage P. The vitamin D content of tissues of rats given ergocalciferol. Biochem J 1954;58(1):172.
38. Girgis CM, Clifton-Bligh RJ, Mokbel N, Cheng K, Gunton JE. Vitamin D signaling regulates proliferation, differentiation, and myotube size in C2C12 skeletal muscle cells. Endocrinology 2014;155(2):347–57.
39. Darr RL, Savage KJ, Baker M, Wilding GE, Raswalsky A, Rideout T, Browne RW, Horvath PJ. Vitamin D supplementation affects the IGF system in men after acute exercise. Growth Horm IGF Res 2016;30–31:45–51.
40. Ellison TI, Dowd DR, MacDonald PN. Calmodulin-dependent kinase IV stimulates vitamin D receptor-mediated transcription. Mol Endocrinol 2005;19(9):2309–19.
41. Cangussu LM, Nahas-Neto J, Orsatti CL, Bueloni-Dias FN, Nahas EA. Effect of vitamin D supplementation alone on muscle function in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial. Osteoporos Int 2015;26(10):2413–21.
42. Ceglia L. Vitamin D and skeletal muscle tissue and function. Mol Aspects Med 2008;29(6):407–14.
43. Foo LH, Zhang Q, Zhu K, Ma G, Trube A, Greenfield H, Fraser DR. Relationship between vitamin D status, body composition and physical exercise of adolescent girls in Beijing. Osteoporos Int 2009;20(3):417–25.
44. Viljakainen HT, Natri AM, Kärkkäinen M, Huttunen MM, Palssa A, Jakobsen J, Cashman KD, Mølgaard C, Lamberg-Allardt C. A positive dose–response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. J Bone Miner Res 2006;21(6):836–44.
45. Berger PK, Pollock NK, Laing EM, Chertin V, Bernard PJ, Grider A, Shapses SA, Ding KH, Isales CM, Lewis RD. Zinc supplementation increases procollagen type 1 amino-terminal propeptide in premenarcheal girls: a randomized controlled trial. J Nutr 2015;145(12):2699–704.
46. Kulkarni B, Kuper H, Kinra S, Charyulu MS, Ben-Shlomo Y, Smith GD, Ebrahim S, Radhakrishna K. Relationship of vitamin D status with muscle mass and muscle strength in young Indian adults–evidence from Andhra Pradesh children and parents study cohort. Eur J Nutr Food Saf 2015;5(5):918–9.