Dietary vitamin D dose-response in healthy children 2 to 8 y of age: a 12-wk randomized controlled trial using fortified foods1,2

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ABSTRACT

Background: Vitamin D is fundamental for bone health. A high proportion of Canadian 2- to 8-y-olds do not meet the Estimated Average Requirement (EAR) of 400 IU/d.

Objective: The objective was to determine whether vitamin D intakes consistent with the EAR or Recommended Dietary Allowance (RDA), through fortification of additional dairy products, would result in higher vitamin D status in young children.

Design: Participants aged 2–8 y (n = 77; Montreal, Canada) were randomly assigned to 1 of 3 dietary vitamin D targets (control; EAR: 400 IU/d; or RDA: 600 IU/d) for 12 wk (January to April 2014). Anthropometric measurements, demographic characteristics, dietary intakes, fasting serum parathyroid hormone, 25-hydroxyvitamin D [25(OH)D], and ionized calcium were compared by using mixed-model ANOVA.

Results: Participants’ mean ± SD age was 5.1 ± 1.9 y; 54.5% were boys with body mass index z scores of 0.50 ± 0.85. Compliance was 85% overall. No differences were observed in baseline dietary vitamin D intakes or serum 25(OH)D. At 12 wk, the EAR and RDA groups had significantly higher vitamin D intakes (median [IQR]: control, 227 [184–305] IU/d; EAR, 410 (363–516) IU/d; and RDA, 554 (493–653) IU/d; P < 0.05) and serum 25(OH)D concentrations (control: 61.4 ± 10.0 nmol/L; EAR: 64.1 ± 12.8 nmol/L; RDA: 63.7 ± 12.4 nmol/L; P < 0.05) than the control group. Ninety-six percent of children in the EAR and RDA groups and 67% of the control group had 25(OH)D concentrations ≥50 nmol/L.

Conclusion: Increasing the vitamin D intakes of young children through fortification of alternative dairy products results in significantly higher serum concentrations of 25(OH)D and a significantly greater proportion of children with serum 25(OH)D ≥50 nmol/L, during periods of minimal ultraviolet B radiation exposure. This trial was registered at clinicaltrials.gov as NCT02097160 and had Health Canada Temporary Marketing Authorization Letters for both products (TM-13-0432 and TM-13-0433).

INTRODUCTION

Vitamin D is a fundamental nutrient for bone mineral accrual (1). Because vitamin D cannot be endogenously synthesized year-round above 40°N latitude because of limited UVB solar radiation from October through March (2), it is considered to be an essential nutrient in the diet (1, 3). Children aged 2–8 y are recommended to daily consume 2–2.5 servings of milk and alternatives by Canada’s Food Guide and MyPlate (4, 5). However, >50% of children in Canada and the United States do not meet the Estimated Average Requirement (EAR)6 for vitamin D on the basis of national surveillance surveys (6, 7). In cross-sectional studies in young children, vitamin D status is significantly higher in the summer months than in the winter months (8–10). To the best of our knowledge, no studies in young children in North America have investigated whether winter-time declines in vitamin D status are significant when followed prospectively. The amount of dietary vitamin D needed to sustain vitamin D status throughout winter in young children is unknown.

Vitamin D fortification policy in Canada includes adding vitamin D to milk and margarine and in the United States includes adding vitamin D to milk and some yogurt and cheese products as well as other foods (11–13). Average fluid milk intake of Canadian 4–8-y-olds was 375–575 mL/d in 2004 (4). NHANES 1999–2002 data show that preschool-aged children have a mean milk intake of 364 mL/d (14), and NHANES 2001–2004 data show that 59.1% ± 3.17% of 2- to 3-y-olds and 57.8% ± 2.14% of 4- to 8-y-olds were meeting the recommended milk intake (15). Meeting the food group recommendation by consuming only fortified fluid milk provides half of the EAR of 400 IU

1 Supported by funding from Dairy Farmers of Canada, the Canadian Foundation for Innovation and Canada Research Chairs, and in-kind support from Agropur and Ultima Foods for the study products.

2 Supplemental Tables 1 and 2 are 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.

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6 Abbreviations used: EAR, Estimated Average Requirement; FFQ, food-frequency questionnaire; ITA, individual typological angle; PTH, parathyroid hormone; RDA, Recommended Dietary Allowance; 25(OH)D, 25-hydroxyvitamin D.
vitamin D/d and one-third of the Recommended Dietary Allowance (RDA) of 600 IU vitamin D/d. These values were set to align with serum 25-hydroxyvitamin D [25(OH)D] concentrations of 40 and 50 nmol/L, respectively (3). This means that children are at risk of not meeting the Institute of Medicine population target of 40 nmol 25(OH)D/L (11). By consuming yogurt or cheese instead of fluid milk, vitamin D intakes may be lower, because these foods contain <30% of the vitamin D that milk contains (3).

Vitamin D fortification regulations were set when recommended intakes were lower (11–13). Thus, the EAR for vitamin D cannot be met by most children who consume currently available marketplace foods. The primary objective of this study was to determine whether vitamin D intakes consistent with the EAR or RDA, through fortification of additional dairy products, would result in higher vitamin D status in young children. The secondary objective was to confirm whether vitamin D intakes that reach the RDA sustain a serum 25(OH)D concentration of 50 nmol/L in young children during winter and early spring months.

METHODS

Subjects

Families and children were recruited from daycare centers within the greater Montreal region. Parents were provided a recruitment letter through registered daycares and contacted the research unit if interested. For those aged >6 y, previous participants of a vitamin D study (8) were invited and media recruitment strategies were also used. Inclusion criteria were as follows: healthy, prepubertal, regularly consuming milk and milk products, within 2 BMI z scores from zero for age and sex on the basis of WHO growth charts (16), and not taking any nutritional supplements. Exclusion criteria included chronic diseases or medications known to affect vitamin D, infections of the immune system, known anemia, small size at birth, or preterm birth at <37 wk of gestation. During screening, if children were taking nutritional supplements, the parents were asked to stop the supplement intake of their children for 1 mo [2 half-lives of 25(OH)D] (17) before the study. Twelve 2- to 3-y-olds and twenty-two 4- to 8-y-olds were taking vitamin D supplements (68% took ≤200 IU/d, 29% took 400 IU/d, and 1 child took 1000 IU/d). There were no differences in supplement intake between groups (Table 1).

Study design

This was a 12-wk double-blind, randomized controlled trial, following the CONSORT (Consolidated Standards of Reporting Trials) statement, with recruitment from 8 January to 16 February 2014. Children were randomly allocated in a 1:1:1 ratio to 1 of 3 groups (Figure 1) in a double-blind fashion stratified by 2-y blocks for age with the use of http://randomization.com. Group codes were randomly chosen by the safety officer for this trial to ensure blinding of researchers. All of the children consumed normally fortified fluid milk ad libitum. The control group consumed two 93-mL drinkable yogurts/d and one 21-g piece of cheddar cheese with no added vitamin D in addition to their regular household meals. The 2 treatment groups consumed the same yogurt beverage and cheese products, but with added vitamin D-3 to reach an estimated total dietary vitamin D intake consistent with the EAR (400 IU/d) and RDA (600 IU/d). It was expected that, on average, the control groups would consume 250 mL milk/d (100 IU vitamin D) and obtain 30 IU vitamin D normally occurring in the yogurt beverage and 10–65 IU from other foods (fish/seafood, margarine, or other products with vitamin D added), providing a total of 140–195 IU vitamin D/d. The yogurt beverage in the EAR group contained a total of 42 IU vitamin D/93 mL and in the RDA group contained a total of 125 IU/93 mL. Both groups consumed 200 IU/d through cheese (200 IU/21 g). The products were provided precoded by the companies, and vitamin D content was verified to be within ±8% by Maxxam Analytique, Inc. (Saint Laurent, Quebec, Canada), for the yogurt and within ±5% by O’Neal Scientific Services, Inc. (St. Louis, Missouri), for the cheese. Families were instructed to otherwise follow their normal lifestyle. Children and families were seen at baseline and at 12 wk. At both visits, fasting blood samples were obtained and anthropometric measures were taken along with surveys on demographic characteristics, sun exposure, physical activity, and dietary intake.

Assessments

Blood sampling, vitamin D status, and parathyroid hormone

Fasting venipuncture samples were taken (0700–1100) to control for diurnal variation; parents were instructed that their child could not eat anything after midnight. At baseline and the end of study, 2 mL whole blood was separated to obtain serum for the measurement of 25(OH)D and parathyroid hormone (PTH), and 0.1 mL whole blood was immediately analyzed for ionized calcium.

Serum total 25(OH)D and intact 1-84 PTH were measured by using an autoanalyzer (Liaison; Diasorin). The sensitivity of these assays was 10 nmol/L for 25(OH)D and 2.36 pg/mL for PTH. With the use of National Institute for Standards and Technology 25(OH)D standard 972a levels 1 and 4, the inter- and intra-assay CVs were 2.1% and 2.2% for level 1 and 5.8% and 6.9% for level 4, with an accuracy of ≥96%. The laboratory also maintains certification with the Vitamin D External Quality Assessment Scheme. PTH controls had inter- and intra-assay CVs of 3.6% and 2.6% for control 1 and 3.7% and 7.2% for control 2, with an accuracy of ≥95% for controls 1 and 2. Ionized calcium was measured immediately in whole blood (0.1 mL) as a safety measure by using a portable blood gas unit (ABL80 FLEX; Radiometer Medical A/S).

Dietary assessment and compliance

A 24-h food intake assessment (day before sampling) was used to assess energy and macronutrient intake. Intake data for 3 d have been shown to be sufficient to estimate energy intake; however, >7 d are needed to estimate calcium intake (18). Therefore, a validated 13-item semiquantitative, 1-mo food-frequency questionnaire (FFQ) was used specifically to estimate calcium and vitamin D (8). The FFQ had 13 questions that asked about intake of milk and alternatives, vitamin D–fortified juices, margarine and baby foods, as well as fish and seafood. Both the FFQ and 24-h assessment were completed by the parents with
TABLE 1
Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>EAR</th>
<th>RDA</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Age, y</td>
<td>5.0 ± 1.8 (2.0–8.0) (^2)</td>
<td>4.9 ± 2.1 (1.9–8.7)</td>
<td>5.3 ± 2.0 (2.1–8.3)</td>
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<tr>
<td>Male sex, n (%)</td>
<td>12 (50)</td>
<td>15 (56)</td>
<td>13 (50)</td>
</tr>
<tr>
<td>College or higher maternal education achieved, n (%)</td>
<td>19 (79)</td>
<td>20 (75)</td>
<td>20 (77)</td>
</tr>
<tr>
<td>Family income, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;$65,000(^6)</td>
<td>14 (58)</td>
<td>14 (52)</td>
<td>15 (58)</td>
</tr>
<tr>
<td>Not disclosed</td>
<td>4 (17)</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>BMI&lt;br&gt;score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.49 ± 0.96(^4)</td>
<td>0.23 ± 0.78</td>
<td>0.55 ± 0.90</td>
</tr>
<tr>
<td>Height, cm</td>
<td>0.07 ± 0.88</td>
<td>−0.12 ± 1.05</td>
<td>0.27 ± 1.00</td>
</tr>
<tr>
<td>BMI</td>
<td>0.63 ± 1.00</td>
<td>0.40 ± 0.58</td>
<td>0.54 ± 0.91</td>
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<tr>
<td>Vitamin D supplements</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Previous use, n (%)</td>
<td>12 (50)</td>
<td>11 (41)</td>
<td>11 (42)</td>
</tr>
<tr>
<td>Dose, IU/supplement</td>
<td>291 ± 115</td>
<td>290 ± 104</td>
<td>291 ± 243</td>
</tr>
<tr>
<td>Frequency, n/wk</td>
<td>5.0 ± 2.3</td>
<td>4.9 ± 2.5</td>
<td>4.5 ± 1.6</td>
</tr>
<tr>
<td>Serum 25(OH)D category, n (%)</td>
<td></td>
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<td></td>
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<tr>
<td>&lt;30 nmol/L</td>
<td>1 (4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30–39.9 nmol/L</td>
<td>0</td>
<td>2 (7)</td>
<td>0</td>
</tr>
<tr>
<td>40–49.9 nmol/L</td>
<td>6 (25)</td>
<td>5 (19)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>50–124.9 nmol/L</td>
<td>17 (71)</td>
<td>20 (74)</td>
<td>22 (85)</td>
</tr>
<tr>
<td>≥125 nmol/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\) EAR, Estimated Average Requirement; RDA, Recommended Dietary Allowance; 25(OH)D, 25-hydroxyvitamin D.

\(^2\) Mean ± SD; range in parentheses (all such values).

\(^3\) Canadian dollars.

\(^4\) Mean ± SD (all such values).

\(^6\) $65,000.

Demographic characteristics, skin pigmentation, and UVB

At baseline, survey data were obtained for child sun exposure as well as on self-reported income and ethnicity to facilitate understanding of the relations between sociodemographic characteristics, skin pigmentation, and baseline vitamin D status. In case of UVB exposure from travel to southern locations, data at baseline and 12 wk were collected with regard to sun exposure during the previous 30 d as a percentage of body surface area exposed (19), frequency of sunscreen use, and total hours spent in direct sunlight per day. At each time point, the sun index was calculated for each child by multiplying the percentage of body surface area exposed by the time spent outside. Skin type at baseline was established with the use of a spectrophotometer (CM-700d/600d; Konica Minolta) by measuring pigmentation 3 times at the inner upper arm for constitutive pigmentation.

Facultative pigmentation at the forehead, mid-forearm, and lower leg was also measured 3 times at each site to estimate recent exposure to UVB radiation (e.g., travel during December break or later during March break). The individual typological angle (ITA) was calculated by using the L* and b* values with the equation from the Commission Internationale de l’Eclairage (20) and classified into 6 skin types on the basis of Fitzpatrick descriptions. Differences between facultative and constitutive values assisted in qualitative assessment of previous UVB exposure (21, 22) at all visits.

Anthropometric measurements

Height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer (Seca 216; Seca Medical Scales and Measuring Systems). Body weight was measured to the nearest 0.5 kg by using a balance-beam scale (Detecto; Webb) with the child wearing light clothing and no shoes. From these values, BMI (in kg/m²) was calculated and then z scores for weight, height, and BMI were calculated with the use of the WHO 2007 growth standards/References for children < or > 5 y (WHO AnthroPlus). At the study endpoint, dual-energy X-ray absorptiometry (Hologic Discovery 4500A QDR series with Apex V13.2 software) was used to scan the whole body to provide total and regional fat mass (kg and %). Children wore standardized clothing, light pants, and a t shirt and were scanned without sedation. Total body and regional fat mass were quantified because regional fat mass in adults and children inversely correlates to vitamin D status (23, 24). Regional adipose tissue was estimated by using subregion analysis of the full torso as well as between the last floating rib and the iliac crests to reflect...
visceral and subcutaneous fat of the abdominal region. Although dual-energy X-ray absorptiometry estimates of trunk fat do not distinguish subcutaneous from visceral fat, values align well with those from computed tomography scans in women and children (25–27). The whole-body scans take 3 min, resulting in a low-dose radiation exposure (<3 μSv).

Ethics

This study was approved by the McGill University Faculty of Medicine Research Ethics Board in accordance with the Tri-Council Policy on ethics (28), Temporary Marketing Authorization letters were obtained from Health Canada for both products (TM-13-0432 and TM-13-0433). All daycare centers agreed in writing to facilitate the study, and all parents or legal guardians provided written informed consent before the study.

Statistical analyses

A sample size estimate of 20 per group was set to enable the detection of clinically meaningful group differences of 20 ± 16 nmol 25(OH)D/L on the basis of a representative sample of preschool-aged children in Montreal (8) at a 5% significance level with 80% power. To account for a 5–10% drop-out rate and the additional possibility of insufficient blood volume sampled, we aimed to recruit 25 participants per group.

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 was assessed by using the Bartlett test. For the primary analysis, mixed-model ANOVA was used to analyze continuous data accounting for fixed effects (sex/age strata, dietary group) and random effects (e.g., demographic characteristics) with post hoc testing where necessary (i.e., 3 age groups or interactions) with the use of Bonferroni correction. Nonnormal data were log-transformed where applicable [e.g., 25(OH)D]. At endpoint, there were not enough children in the vitamin D status categories of 30–39.9 and 40–49.9 nmol serum 25(OH)D/L to enable reliable statistical analyses; thus, these were combined to form a category of 30–49.9 nmol/L. Less than 5% of data were missing, and imputation approaches were not sought. Compliance on the basis of daily check sheets was compared among groups by using mixed-model ANOVA. Chi-square or Fisher’s exact testing was used for proportions. Linear regression analysis was used to explore correlations between serum 25(OH)D concentration and predictor variables. The model was
constructed on the basis of inclusion of predictor variables vitamin D intake, age, BMI z score, and sex; single variables were added to the model that were significant or that improved the model ($R^2 > 2\%$). The models were checked for prespecified interactions, and if interactions were present, interaction terms were included in the final model. Visual examination was used to test for normality of the residuals. Data are presented as mean ± SDs or medians (IQRs) depending on normality for continuous data or as proportions for ordinal data (e.g., sex). $P < 0.05$ was accepted as significant.

RESULTS

Demographic characteristics

At baseline, children were 5.1 ± 1.9 y old (range: 1.9–8.7 y old), with 54.5% (42 of 77) being male and 72.7% (56 of 77) white. The majority of children were born to mothers who were well educated, with 77% achieving college or university education; household income was >$65,000 in 56% of families, with 9% not disclosing income. No differences in these characteristics were observed between allocation groups. Physical activity was not different between groups at baseline. On weekdays and weekends, 91% and 82% of children, respectively, were very active for ≥60 min.

At baseline, the BMI-for-age z score was 0.51 ± 0.89 overall, with no differences between boys and girls (0.48 ± 0.82 and 0.55 ± 0.97, respectively). The mean height-for-age z score was 0.10 ± 0.98 and the weight-for-age z score was 0.43 ± 0.93. No differences in anthropometric measurements were observed between groups (Table 1). Height velocity was 0.6 ± 0.3 cm/mo and weight velocity was 0.2 ± 0.2 kg/mo over the study period, with no differences between groups (Supplemental Table 1). At the end of the study, there were no significant differences between study groups in body fat percentage (range: 15.1–39.7%); however, the mean android and gynoid fat percentages (android: boys, 20.5% ± 6.1%; girls, 25.1% ± 5.5%; gynoid: boys, 33.0% ± 4.8%; girls, 41.0% ± 8.0%).

Seventy-four (96.1%) children completed the study over 12.1 (range: 10.9–13.9) wk. Twenty-two participants had their 12-wk follow-up in early May instead of April because of rescheduling. Over the course of the study, the median (IQR) durations of childhood illnesses (e.g., common colds) per group were as follows: control, 4.0 (3.0–5.5) d; EAR, 5.0 (2.9–9.3) d; and RDA, 4.5 (3.0–6.0) d. There were no differences between groups. Mean compliance for the study yogurt and cheese products was 86% ± 20% and 84% ± 17%, respectively. For the first week of the study, compliance was 95% ± 12% and 91% ± 18% and decreased to 79% ± 28% and 75% ± 29%, respectively, by the 12th wk. Compliance was significantly different ($P = 0.04$) between 2 to 3 y olds and 4 to 8 y olds for cheese (76% ± 21% compared with 88% ± 13%) but was not different for yogurt (84% ± 19% compared with 88% ± 19%). Overall, the EAR group had significantly lower compliance ($P = 0.02$) for both products (yogurt: 80% ± 22%; cheese: 79% ± 17%) than did the control (yogurt: 89% ± 19%; cheese: 88% ± 13%) and RDA (yogurt: 89% ± 16%; cheese: 84% ± 19%) groups.

Dietary characteristics

The consumption of study products during the trial did not cause a significant change in total energy intake (baseline: 1520 ± 423 kcal/d; 12 wk: 1538 ± 390 kcal/d), protein intake (baseline: 64 ± 23 g/d; 12 wk: 67 ± 24 g/d), fat intake (baseline: 49 ± 19 g/d; 12 wk: 54 ± 19 g/d), or carbohydrate intake (baseline: 212 ± 64 g/d; 12 wk: 201 ± 54 g/d). The children in all groups, on average, met or exceeded the recommended servings of milk and alternatives on the basis of Canada’s Food Guide (Figure 2, Supplemental Table 2). There were no differences in servings of total milk and alternatives consumed (2–3 y: 2.0 ± 1.4 servings/d; 4–5 y: 2.6 ± 1.2 servings/d; and 6–8 y: 2.3 ± 1.3 servings/d) between the 3 age strata. At baseline, the median (IQR) vitamin D intake from the 30-d FFQ of 198 (155–291) IU/d was below the EAR, with no differences between groups or between age strata (Table 2). The vitamin D intake of the control group did not significantly change throughout the study. According to the 30-d FFQ, none of the control group reached 400 IU/D. The percentages of children in the EAR and RDA groups who reached their targets at 12 wk were 60% and 42%, respectively, according to the 30-d FFQ (Table 2). If compliance had been 100%, median (IQR) vitamin D intakes for the EAR and RDA groups would have been 458 (390–509) and 629 (578–698) IU/d. Total milk product intake did not differ over time or between groups (Figure 2). Calcium intake was similar across groups at baseline (control: 1092 ± 367 mg/d; EAR: 1042 ± 429 mg/d; RDA: 1039 ± 418 mg/d) and did not change over the 12 wk, with 61% of all participants reaching the RDA at baseline and 64% reaching the RDA at 12 wk.

Sun exposure

Fifty-six percent (43 of 77) children had Fitzpatrick skin types I, II, or III; 44% (34 of 77) had skin types IV, V, or VI. Only 4%...
Biochemical assessments

With regard to our primary analysis, at baseline, 77% (59) of participants had serum 25(OH)D concentrations between 50 and 125 nmol/L, none had concentrations ≥125 nmol/L, and only 1 participant had a 25(OH)D concentration <30 nmol/L (Table 1). By 12 wk, no children had serum 25(OH)D concentrations <30 nmol/L, and 8 children in the control group and 1 child in each of the EAR and RDA groups had serum 25(OH)D concentrations <50 nmol/L. No child had serum 25(OH)D concentrations ≥125 nmol/L.

At 12 wk, both the EAR and RDA allocation groups had significantly higher 25(OH)D concentrations compared with the control group (Figure 3A) and had a significantly greater change over time in 25(OH)D concentration (Figure 3B). In all of the groups, change in vitamin D status was not different according to age group (P = 0.499). At baseline, all of the groups had similar proportions of children with 25(OH)D above the target concentration consistent with the EAR (96%); RDA: 93%; EAR: 71%; RDA: 74%; EAR: 85% of 50 nmol/L. By 12 wk, 96% of the EAR and RDA groups had serum 25(OH)D concentrations ≥50 nmol/L, which was significantly different compared with the control group [67% with 25(OH)D ≥50 nmol/L]. At 12 wk, 100% of the EAR group and 92% of the control group had 25(OH)D concentrations ≥40 nmol/L.

On the basis of regression analysis (R² = 0.73), 25(OH)D increased significantly (P < 0.05) from Fitzpatrick skin type I–III to IV–VI, by 5.5 (95% CI: 1.1, 10.0) nmol/L, but did not vary significantly on the basis of other variables in the model, BMI z score, total body fat percentage (total or android and gynoid), or number of days in the study. With the use of the same model, for every 100-IU increase in vitamin D intake, serum 25(OH)D increased by 1.7 (95% CI: 0.6, 2.8) nmol/L.

Ionized calcium showed a group by time interaction (P = 0.027), suggesting that values declined in the control group by 12 wk (baseline: 1.31 ± 0.05 mmol/L; 12 wk: 1.28 ± 0.06 mmol/L), but this did not reach significance (P = 0.059), with no differences in the EAR or RDA groups. Average ionized calcium (1.30 ± 0.04 mmol/L) was within normal limits (1.15–1.38 mmol/L).

### TABLE 2
Vitamin D intakes based on a 30-d food-frequency questionnaire

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Fluid milk</th>
<th>Yogurt</th>
<th>Cheese</th>
<th>Other</th>
<th>Total</th>
<th>Met EAR, %</th>
<th>Met RDA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24</td>
<td>161 (117–206)</td>
<td>17 (14–22)</td>
<td>2 (0–3)</td>
<td>76 (50–201)</td>
<td>207 (169–383)</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>EAR</td>
<td>27</td>
<td>173 (106–232)</td>
<td>18 (13–28)</td>
<td>3 (2–4)</td>
<td>77 (53–105)</td>
<td>218 (145–294)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>RDA</td>
<td>26</td>
<td>120 (97–215)</td>
<td>12 (6–18)</td>
<td>2 (1–3)</td>
<td>72 (48–99)</td>
<td>166 (148–306)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td><strong>12 Weeks</strong></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>24</td>
<td>132 (100–160)</td>
<td>40 (36–44)</td>
<td>1 (0–2)</td>
<td>64 (36–95)</td>
<td>227 (184–305)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>EAR</td>
<td>25</td>
<td>121 (66–206)</td>
<td>77 (69–85)</td>
<td>161 (141–213)</td>
<td>53 (28–81)</td>
<td>410 (363–516)</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>RDA</td>
<td>25</td>
<td>112 (74–163)</td>
<td>223 (203–263)</td>
<td>167 (134–202)</td>
<td>67 (30–99)</td>
<td>554 (493–653)</td>
<td>80</td>
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</tbody>
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Values are medians; IQRs in parentheses. Values with different superscript letters are significantly different (P < 0.05) from all other groups with the use of mixed-model ANOVA with Bonferroni post hoc testing, accounting for age, sex, ethnicity, and number of days between baseline and follow-up. EAR, Estimated Average Requirement; RDA, Recommended Dietary Allowance.

**FIGURE 3** Serum 25(OH)D concentrations at baseline and at 12 wk (A), change in 25(OH)D concentrations over time (B), serum PTH concentrations at baseline and at 12 wk (C), and change in PTH concentrations over time (D). An intent-to-treat analysis mixed-model ANOVA [adjusted for age, sex, ethnicity, baseline 25(OH)D, and number of days between baseline and follow-up] with Bonferroni post hoc testing was used. Serum 25(OH)D data were log-transformed before analysis and are presented as unadjusted means ± SDs. **Different from control at 12 wk, P < 0.05; ****different from control at 12 wk and all groups at baseline, P < 0.05. EAR, Estimated Average Requirement; PTH, parathyroid hormone; RDA, Recommended Dietary Allowance; 25(OH)D, 25-hydroxyvitamin D; Δ, change.

(3 of 74) of children traveled to warm countries during the study period, and none of them had changes in ITA from baseline to follow-up. Accordingly, there was no significant tanning of skin from UVB solar radiation. The mean change in ITA over the study on the 3 tanning sites (forehead, lower leg, and lower forearm) was 1.84 ± 3.75 and was not significantly different from zero or significantly different between groups. Participants who had their 12-wk follow-up in May (n = 22) did not have a significantly different mean change in ITA (0.85 ± 2.81) from those with follow-ups in April (2.28 ± 4.06).
in all groups; however, 1 child in the EAR group had a low value (1.10 nmol/L) at 12 wk. The pattern in PTH was the opposite of that of 25(OH)D, although with no significant differences between groups (Figure 3) and an overall mean change in PTH from baseline to 12 wk of \(-0.5 \pm 5.1 \text{ pg/mL}\). Time of blood draw in the morning did not have an effect on PTH concentration \((P = 0.37)\). One child in the EAR group (6.3 pg/mL) at baseline and 4 children in the EAR group and 2 children in the RDA group (7.4–8.8 pg/mL) at 12 wk had PTH values below the normal range (9–60 pg/mL).

**DISCUSSION**

The primary objective of this study was to establish whether vitamin D intakes consistent with the EAR or RDA, through fortification of additional dairy products, would result in higher vitamin D status than with normal vitamin D intakes. Although we observed a significant difference in dietary intake, not all of the children were able to meet the EAR and RDA targets. Because the EAR is set to be a sufficient for 50% of individuals, and 60% of our EAR group met this target, we would have expected more than half of the children in this group to maintain the concentration of 40 nmol 25(OH)D/L, whereas almost all (96%) children maintained this value. For the RDA group, the design was intended to elevate usual intakes to meet the RDA over the course of the study, but this did not appear to be achieved on the basis of the 30-d FFQ. In the real-life situation of our study, children had a median (IQR) of 4.5 (3.0–6.0) d of illness, which contributed to intakes below the RDA as captured in the FFQ. Nonetheless, all but 1 child was able to achieve and maintain a 25(OH)D concentration of \(\geq 50 \text{ nmol/L}\). These results suggest that the baseline vitamin D status observed in our study was sufficient to protect against 25(OH)D falling below 40 nmol/L or that the EAR is higher than necessary for the needs of young children. Overall, the significantly increased vitamin D consumption in the fortified groups compared with the control group led to significantly higher serum 25(OH)D concentrations in the winter and early spring months.

Interestingly, the RDA intake group (64.1 ± 10.0 nmol/L) in our study did not have significantly higher serum 25(OH)D compared with the EAR group (63.7 ± 12.4 nmol/L). Both the EAR and RDA groups also had 96% of children with 25(OH)D concentrations \(\geq 50 \text{ nmol/L}\). The EAR and RDA recommendations from the Institute of Medicine are mostly based on randomized controlled trials in adults from northern Europe (3), which may explain why significant decreases in PTH concentration were not seen in the fortified groups in our study. The lower PTH is likely due to the very good calcium intakes in the present study. Ionized calcium, being similar between groups and unchanged over the 12-wk study period, shows the safety of fortifying dairy products with vitamin D in an effort to reach EAR and RDA targets.

To our knowledge, our study methodology presented a novel fortification model for children by using 2 fortified dairy products to augment usual intakes. Increased daily intake of vitamin D was chosen because it is more efficient at maintaining or increasing serum 25(OH)D than are large monthly (37) or seasonal (38) intakes. Another strength of this design was that it allowed children to maintain day-to-day consumption of dairy products, with the study products replacing those normally consumed (Figure 2, Supplemental Table 2). Allowing normal food intake habits for children likely contributed to our high level of overall compliance (84–86%). Poor compliance has been observed when children have difficulty meeting food intake or nutrient intake, there would be a resulting increase of 1.2 (95% CI: 0.72, 1.68) nmol/L (33). A trial in adults aged \(\geq 50 \text{ y} (n = 56)\) in Ireland through 10 wk in winter showed a 2.5-nmol/L increase for every 100-IU increase in vitamin D intake (34). However, previous trials in children that ran from September to April (33) and from January to March (34) showed that each 100-IU/d increase in vitamin D intake led to a 7- to 10-nmol/L increase in serum 25(OH)D. In our study, on the basis of linear regression analysis, each 100-IU/d increase in vitamin D intake through fortified foods resulted in an average increase in 25(OH)D of 1.7 (95% CI: 0.6, 10.0) nmol/L. Our results may differ from previous work in children due to our study taking place over only 12 wk, beginning in the middle of winter. Longitudinal results from adults in Denmark (14) showed a mean decrease of 25 nmol 25(OH)D/L between October and December and of 4 nmol/L between January and March, which is similar to our results in young children. A larger increase in 25(OH)D may have been observed had baseline vitamin D status been deficient, as shown in young children in Mongolia (16).

When 25(OH)D is low, it is common to observe elevated PTH (35). At baseline, PTH (15.6 ± 4.8 pg/mL) values in our study were similar to those in 4- to 8-y-old children after supplementation with 1000 IU vitamin D-3/d (13.3 ± 7.6 pg/mL) (36), which may explain why significant decreases in PTH concentration were not seen in the fortified groups in our study. The lower PTH is likely due to the very good calcium intakes in the present study. Ionized calcium, being similar between groups and unchanged over the 12-wk study period, shows the safety of fortifying dairy products with vitamin D in an effort to reach EAR and RDA targets.
intake goals (39). Although our compliance declined by the end of the study, a higher level of compliance may have been observed with the availability of multiple yogurt flavors and types of cheese because parents may not have to put as much pressure on children to eat the foods. In an American study (n = 27 children; mean ± SD age: 4.0 ± 1.0 y), pressure by parents on a child to eat a food was shown to significantly decrease the intake of that food by 20% and to result in children vocalizing 5 times as many negative comments about the food (40).

Limitations of our study include that the data are not representative of all young Canadian children because a large proportion of the parents had a university education and households had a median family income above the average for Quebec and Canada (41). Also, it is possible that dietary vitamin D intakes were underestimated because 25(OH)D present in animal-based foods was not included in our total dietary intake calculation. On the basis of NHANES data (n = 8579; 2007–2008), contributions from other foods are estimated to be 68–116 IU vitamin D/d in the diets of persons aged ≥2 y (42). Last, on the basis of previous research, it was anticipated that baseline 25(OH)D values may differ between white and nonwhite children (43) and that the effect of vitamin D food fortification on serum 25(OH)D would not differ between white and nonwhite children (44). We detected a significant difference at baseline and wk in serum 25(OH)D between Fitzpatrick skin types I–III compared with IV–VI but could not further analyze differences between all 6 skin types due to a limited sample size.

In conclusion, young children who consumed vitamin D–fortified yogurt and cheese products had significantly higher vitamin D intakes and improved vitamin D status over the winter and spring periods studied. These results show a need for future studies to confirm the vitamin D EAR of 400 IU/d for young children on the basis of 25(OH)D response and functional outcomes.

We thank Sandra Dell’Elce for help with procuring blood samples and McGill University graduate students Laura Plante and May Slim and undergraduate students Ziwei Zheng and Zhaorong Wang for help with dietary assessment and analysis. Ms. Plante and Ms. Slim received financial remuneration for their assistance in dietary assessment.

The authors’ responsibilities were as follows—NRB, PL, SA, CAV, and HAW: acquisition of data and administrative, technical, or material support; HAW: full access to all of the data in the study and responsibility for the integrity of the data and the accuracy of the data analysis; JLM, FR, and HAW: study concept and design and obtained funding; NRB, CAV, and HAW: analysis and interpretation of data and drafting of the manuscript; NRB and HAW: statistical analysis; SA, CAV, and HAW: study supervision; and all authors: critical revision of the manuscript for important intellectual content and reading and approval of the final manuscript. JLM and HAW are members of the Dairy Farmers of Canada Expert Scientific Advisory Committee. Dairy Farmers of Canada did not have a role in design, implementation, analysis, or interpretation. None of the authors had a conflict of interest.

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