Vitamin D-Fortified Milk Achieves the Targeted Serum 25-Hydroxyvitamin D Concentration without Affecting That of Parathyroid Hormone in New Zealand Toddlers1–3

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Abstract

For young children, the level of vitamin D required to ensure that most achieve targeted serum 25-hydroxyvitamin D \[25(\text{OH})D\] \(\geq 50\) nmol/L has not been studied. We aimed to investigate the effect of vitamin D-fortified milk on serum 25(OH)D and parathyroid hormone (PTH) concentrations and to examine the dose–response relationship between vitamin D intake from study milks and serum 25(OH)D concentrations in healthy toddlers aged 12–20 mo living in Dunedin, New Zealand (latitude 46°S). Data from a 20-wk, partially blinded, randomized trial that investigated the effect of providing red meat or fortified toddler milk on the iron, zinc, iodine, and vitamin D status in young New Zealand children \((n = 181;\text{ mean age 17 mo})\) were used. Adherence to the intervention was assessed by 7-d weighed diaries at wk 2, 7, 11, 15, and 19. Serum 25(OH)D concentration was measured at baseline and wk 20. Mean vitamin D intake provided by fortified milk was 3.7 mg/d (range, 0–10.4 mg/d). After 20 wk, serum 25(OH)D concentrations but not PTH were significantly different in the milk groups. The prevalence of having a serum 25(OH)D \(\geq 50\) nmol/L remained relatively unchanged at 43% in the meat group, whereas it significantly decreased to between 11 and 15% in those consuming fortified study milk. In New Zealand, vitamin D intake in young children is minimal. Our findings indicate that habitual consumption of vitamin D-fortified milk providing a mean intake of nearly 4 mg/d was effective in achieving adequate year-round serum 25(OH)D for most children. J. Nutr. 141: 1840–1846, 2011.

Introduction

In unsupplemented populations, most circulating 25(OH)D is derived from vitamin D produced in the skin following exposure to UVB radiation. However, at high latitudes both north and south of the equator, the decline in UVB intensity during the winter months results in seasonal fluctuations in serum 25(OH)D concentrations (1). Consequently, the prevalence of low vitamin D status defined as a 25(OH)D level below the desirable bone health target of 50 nmol/L (1) is increasingly documented among children and adolescents (2–13).

1 Supported by the Health Research Council of New Zealand, Meat and Livestock Australia, Meat and Wool New Zealand, and the University of Otago. Heinz Wattie’s New Zealand Ltd provided the micronutrient-fortified milk. Fonterra New Zealand Ltd provided the vitamin D-fortified milk.
3 This study was registered at www.actr.org.au as ACTRN12605000487617.
4 Abbreviations used: EAR, Estimated Average Requirement; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
5 To whom correspondence should be addressed. E-mail: lisa.houghton@otago.ac.nz.

Food fortification and/or supplementation strategies to meet the seasonal reliance on dietary sources of vitamin D may be necessary to prevent cyclical decreases in vitamin D status. However, the question arises as to how much oral vitamin D is required to maintain year-round serum 25(OH)D concentrations. The Food and Nutrition Board of the Institute of Medicine recently revised the EAR and RDA for vitamin D using a simulated intake-response relationship based on serum 25(OH)D concentrations indicative of bone health benefits and maximal calcium absorption (1,14). For children and adolescents aged 1–18 y, a concentration of 40 nmol/L (from the middle of the range of 30–50 nmol/L at which risk to the population increases) was selected to serve as the target concentration for the EAR (i.e. meeting the needs of ~50% of the population) and 50 nmol/L for the RDA (i.e. covering the needs of 97.5% of the population) (1). The DRI Committee then used data from 9 vitamin D intervention studies (15–23) to establish regression equations of the stimulated response of serum 25(OH)D concentrations to total vitamin D intake. Only one of these studies was conducted in children and adolescents (aged 6–14 y) (17) and no data were available for toddlers. Moreover, considerable uncertainties in
the simulated dose–response relationship were acknowledged, particularly in relation to the widths of the CI.

Randomized controlled trials are needed to further elucidate the intake-response relation of vitamin D on serum 25(OH)D and health outcomes in young children. New Zealand is an ideal country to study the impact of dietary vitamin D, because intake levels in children are low and the fortification of foods is limited both in amount and distribution. Thus, the purpose of our study was to investigate the effect of vitamin-D fortified milk on serum 25(OH)D and PTH concentrations and to examine the dose–response relation between dietary intake and serum 25(OH)D concentrations in a healthy population of young children aged 12–20 mo living in Dunedin, New Zealand (latitude 46°S).

Participants and Methods

Participants and study design. This study used data from the Toddler Food Study, a 20-wk, partial, double-blind (milk groups blinded), randomized intervention trial conducted from February 2004 to December 2005 in Dunedin, New Zealand that investigated the effect of providing red meat or powdered fortified toddler milk on the biochemical iron status of healthy, nonanemic children aged 12–20 mo (24). Sample size was determined by power calculation based on the expected change in the prevalence of low iron status as described in detail elsewhere (24). Here, we present data related solely to the effect of fortified toddler milk on a principal secondary endpoint, vitamin D status.

Two-hundred and twenty-five toddlers were recruited from Dunedin, New Zealand and the surrounding areas (latitude 46°S). This region has a temperate climate with a summer mean temperature of 14°C and a winter mean temperature of 5°C with mean sunshine hours in the winter ranging from 98 to 122 h/mo (25). The nadir in UV radiation occurs midwinter (July) after the peak 6 mo earlier in summer (December). UVB wavelengths are present when the UV index is >3, and thus cutaneous production of vitamin D would be limited between the months of April and August.

The inclusion criteria for participation were that the child was apparently healthy and 12–20 mo of age inclusive, not currently consuming a fortified milk, and that the caregivers were willing for their child to be randomized to either a meat or a milk intervention. Toddlers were excluded if they had a baseline hemoglobin concentration <105 g/L or a baseline hemoglobin concentration <110 g/L and serum ferritin <12 µg/L. On enrollment, each primary caregiver completed a self-administered sociodemographic questionnaire. Toddlers were then randomized into a red meat intervention group (n = 90), a micronutrient-fortified cow milk powder group (n = 45) (Heinz Nurture Toddler Enriched Milk Drink; Heinz Wattie's), or a whole cow milk powder fortified with vitamin D (n = 90) (Standard Instantized Whole Milk Powder with required A and D added; Fonterra). Nonfasting peripheral venipuncture blood samples were collected at baseline and postintervention and measurements of weight and length were taken according to standardized procedures (26). Ethical approval was obtained from the Human Ethics Committee of the University of Otago, Dunedin, New Zealand, and written informed consent was obtained from each child’s primary caregiver.

Participants in the milk groups were instructed to replace their regular milk with the assigned study milk. For the meat group, caregivers were provided toddler-friendly meat dishes and asked to offer their toddler at least 2 portions of meat (~28 g red meat/portion). The nutrient composition of the commercially available study milks are presented in Table 1. The vitamin D concentration of the study milks was independently confirmed by laboratory analysis (New Zealand Laboratory Services). The weighted mean analyzed vitamin D concentration of the study milks were: micronutrient-fortified milk, 6.3 µg cholecalciferol/100 g powder; and vitamin D-fortified whole milk, 6.0 µg cholecalciferol/100 g powder.

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TABLE 1 Nutrient composition of the vitamin D-fortified and micronutrient-fortified study milks

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Vitamin D-fortified milk1</th>
<th>Micronutrient-fortified milk2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Powder</td>
<td>Prepared drink3</td>
</tr>
<tr>
<td>Energy, KJ</td>
<td>2020</td>
<td>278</td>
</tr>
<tr>
<td>Protein, g</td>
<td>285</td>
<td>3.9</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>26.4</td>
<td>3.6</td>
</tr>
<tr>
<td>SFA, g</td>
<td>16.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>33</td>
<td>4.6</td>
</tr>
<tr>
<td>Cholecalciferol, µg</td>
<td>6.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>960</td>
<td>132</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Iodine, µg</td>
<td>40.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

1 Based on 2004 New Zealand Food Composition Tables unless otherwise stated.
2 Obtained from the manufacturer’s label (Heinz Nurture Toddler Enriched Milk Drink; Heinz Wattie’s) unless otherwise stated.
3 100 g prepared drink is 13.8 g of powder and 88.3 g of water.
4 100 g prepared drink is 15.3 g of powder and 84.7 g of water.
5 Analyzed by New Zealand Laboratory Services.
6 Analyzed in the Department of Human Nutrition’s trace element laboratory, University of Otago, Dunedin, New Zealand.
7 Analyzed by RJ Hill Laboratories, New Zealand.

Dietary assessment. Adherence to the intervention was assessed by asking each caregiver to record the gram weight of study meat or powdered milk (and water used to make the milk drink) including any leftover for 7 consecutive days at wk 2, 7, 11, 15, and 19 of the 20-wk intervention period. The estimated amount of vitamin D consumed from study milk was calculated by multiplying the amount of powder consumed (g) × µg vitamin D/g study milk for each batch recorded as used by the caregiver. Total dietary calcium intake data were collected using 3-d food diaries at baseline and at wk 4 and 18. Participants were instructed by trained nutritionists or a registered dietitian on how to weigh and record all food and beverages consumed over 3 randomly assigned consecutive days using a dietary scale (Vista Electronic Kitchen Scale, Model 3010, Salter Housewares; precision ± 1 g). Dietary calcium intakes were analyzed using the software program Diet Cruncher 1999–2001, version 1.2.0 (27) with the New Zealand Food Composition Database (28).

Biochemical analyses. Blood samples were collected into a vacutainer (Becton Dickinson) with no anticoagulant, refrigerated immediately after collection, and processed within 2 h. The serum was kept frozen at −80°C until 2008 and 25(OH)D was measured in duplicate by RIA (Diasorin) with an analytic sensitivity of 4 nmol/L. The control samples provided by the manufacturer were within the recommended range and the inter-assay CV based on a pooled serum was 13% (n = 5). Aliquots for serum PTH were frozen only once and concentrations were measured as single samples using an electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics). The PTH assay showed a detection sensitivity of 1.2 ng/L and its intra-assay and inter-assay CV were <6%. To avoid between-run variation, pretreatment and posttreatment samples from each participant were assayed in one batch at the end of the study.

Statistical analysis. Linear mixed models with a random participant effect were used to compare serum 25(OH)D and PTH concentration between the 3 arms of the trial in terms of changes from baseline through a group-by-time interaction. Due to the important effect of season, which generally differed at both time points, a model examining follow-up values controlling for baseline values was not used. Instead, season was controlled for at both time points. Log-transformations were used where this improved the distribution and/or homoscedasticity of model residuals. Where log-transformations were used, results are shown as ratios of geometric means rather than differences of arithmetic means.

There was some variation in vitamin D content between batches due to the manufacturing process. The weighted means are based on the total quantity of each batch recorded as used by caregivers for that milk type.
We further categorized vitamin D status as 25(OH)D concentrations <50 nmol/L and 25(OH)D <75 nmol/L, and then compared changes of these 2 statuses using logistic mixed models as above. To determine the serum 25(OH)D response to the amount of vitamin D consumed, a linear mixed model was used to assess the effect of vitamin D intake received from the vitamin D-fortified milks with a season-by-dose interaction used to investigate different effects during different seasons. Models were created for each outcome [serum 25(OH)D concentration, PTH concentration] looking at the effects of season (4 categories based on solstice and equinox dates), child sex, child age at baseline, breastfeeding (no/yes for any breastfeeding at baseline), parental education (no/yes for tertiary education), and second-hand smoke exposure (no/yes for any) in a model containing the group, time, and a group-by-time interaction. Dietary calcium was an additional independent variable used for PTH only and vitamin D dose and dose-by-season were used for the response model only. Independent variables with \( P < 0.25 \) when modeled separately were included in the final model for each outcome. Interactions between each of the predictors in these final models and time were tested for and retained where significant. To determine if associations with continuous independent variables might be nonlinear, fractional polynomial regression was used to assess nonlinearities in a linear regression model using Huber-White SE to account for repeated measures. Where changes were normally distributed, paired \( t \) tests were used to test for differences between baseline and wk-18 dietary calcium intake. Values in the text are presented as geometric mean (95% CI). All analyses were conducted in Stata 11.1 with a 2-sided 0.05 level of significance used.

Results

Of the 225 children randomized to the study, preintervention serum 25(OH)D were available for a total of 181 participants (\( n = 74 \) in the meat group, \( n = 72 \) in the vitamin D-fortified milk group, \( n = 35 \) in the micronutrient-fortified milk group). The mean age of the toddlers at study entry was 17 mo, the majority were New Zealand Europeans, and length and weight were age appropriate. None of the children were taking supplemental vitamin D at the time of the study. The mean 25(OH)D concentration at baseline was 52.3 (95%: 48.9, 55.9) nmol/L. Seventy-nine percent of all participants (\( n = 143 \) of 181) had a 25(OH)D concentration <75 nmol/L, 45% (\( n = 82 \) of 181) had 25(OH)D <50 nmol/L, and 11% (\( n = 20 \) of 181) had 25(OH)D <30 nmol/L.

During the intervention, mean (95% CI) intake of study milk powder was 63.2 (46.2, 86.6) g/d in the micronutrient-fortified milk group, providing a mean dietary intake of vitamin D of 3.9 (2.8, 5.4) \( \mu \)g/d. Similarly, the vitamin D-fortified, whole milk group was consuming an estimated 59.6 (52.8, 67.4) g powder/d with a corresponding value for dietary vitamin D intake of 3.6 (3.2, 4.2) \( \mu \)g/d. The estimated mean grams of milk powder consumption and dietary intake of vitamin D were similar across study milk groups. The range of dietary vitamin D intakes as provided by the study milk was 0–10.4 \( \mu \)g/d. Only 2 participants consumed >10 \( \mu \)g/d set as the EAR and no participants had intakes that met the RDA of 15 \( \mu \)g/d (1). Postintervention median (25th and 75th percentiles) dietary calcium intake of all participants was 752 (364, 962) mg/d. Median calcium intakes decreased in the meat group from 785 mg/d at baseline to 687 mg/d at wk 18 (\( t \) test for changes, \( P = 0.009 \)); however, the median calcium intake for the meat group was well above the EAR of 500 mg/d for children age 1–3 y (1).

After 20 wk of intervention, there was evidence of a difference in changes for mean serum 25(OH)D concentrations between groups. Relative to the meat group, mean serum 25(OH)D concentrations significantly increased in the vitamin D-fortified whole milk group and the micronutrient-fortified milk group (Table 2). There was no evidence of a difference between the 2 milk groups in the observed 25(OH)D increases (\( P = 0.46 \)). Despite significantly higher serum 25(OH)D concentrations in the milk groups at 20 wk, there was no evidence of differences between groups in the change in PTH concentrations (group-by-time interaction, \( P = 0.82 \)). Furthermore, the prevalence of having a serum 25(OH)D concentration <75 nmol/L did not differ among the 3 treatment groups (\( P = 0.71 \) (Table 3)). However, the prevalence of having a serum 25(OH)D <50 nmol/L differed between groups (group-by-time interaction, \( P = 0.010 \)), remaining relatively unchanged in the meat group (\( P = 0.24 \)), and substantially decreased in both the vitamin D-fortified whole milk group (\( P = 0.017 \) compared to the meat group) and the micronutrient-fortified milk group (\( P = 0.008 \) compared to the meat group). The number of participants with a serum 25(OH)D <30 nmol/L at the end of the 20-wk intervention remained relatively unchanged from baseline in the meat group (12%; \( n = 9 \) of 74) yet declined in the study milk groups to 3% (vitamin D-fortified milk, \( n = 2 \) of 72; micronutrient-fortified milk, \( n = 1 \) of 35) (data not shown). The smaller number of participants classified using this lower threshold did not allow for statistical analysis.

In the meat group, postintervention seasonal mean serum 25(OH)D concentrations were 31 nmol/L lower in the winter months of June, July, and August (\( n = 22 \)) compared to the summer months (December, January, and February) (\( n = 4 \)) (Fig.

<table>
<thead>
<tr>
<th>Measurement and treatment group</th>
<th>( n )</th>
<th>Baseline</th>
<th>wk 20</th>
<th>Difference in change relative to meat group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25(OH)D (^1), nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>74</td>
<td>48.8 (44.6, 53.1)</td>
<td>52.6 (48.0, 57.2)</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin D-fortified milk</td>
<td>72</td>
<td>52.8 (48.1, 57.4)</td>
<td>70.5 (64.3, 76.8)</td>
<td>24.1 (7.6, 43.2)*</td>
</tr>
<tr>
<td>Micronutrient-fortified milk</td>
<td>35</td>
<td>48.9 (42.9, 55.0)</td>
<td>69.8 (61.2, 78.5)</td>
<td>32.5 (11.3, 57.8)*</td>
</tr>
<tr>
<td>Serum PTH (^2), ng/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>74</td>
<td>6.7 (5.0, 8.3)</td>
<td>7.5 (5.6, 9.4)</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin D-fortified milk</td>
<td>72</td>
<td>6.0 (4.5, 7.6)</td>
<td>6.3 (4.6, 7.9)</td>
<td>—</td>
</tr>
<tr>
<td>Micronutrient-fortified milk</td>
<td>35</td>
<td>6.1 (3.7, 8.4)</td>
<td>6.1 (3.7, 8.5)</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\) 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

\(^2\) Adjusted geometric means (95% CI) from model controlling for season and parental sex, education, and smoking. Significant time \( \times \) group interaction \( (P = 0.001) \). *Different from the meat group, \( P < 0.01 \).

\(^3\) Adjusted geometric means (95% CI) from model controlling for season, age, breastfeeding, parental smoking, and dietary calcium intake.
TABLE 3  Prevalence of serum 25(OH)D <75 nmol/L in toddlers after a 20-wk intervention with red meat, vitamin D-fortified milk, or micronutrient-fortified milk

<table>
<thead>
<tr>
<th>Treatment</th>
<th>25(OH)D &lt;75 nmol/L</th>
<th>25(OH)D &lt;50 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Baseline wk 20</td>
</tr>
<tr>
<td>Meat</td>
<td>74</td>
<td>82 (61)</td>
</tr>
<tr>
<td>Vitamin D-fortified milk</td>
<td>72</td>
<td>74 (53)</td>
</tr>
<tr>
<td>Micronutrient-fortified milk</td>
<td>35</td>
<td>83 (29)</td>
</tr>
</tbody>
</table>

1 25(OH)D, 25-hydroxyvitamin D.
2 OR (95% CI) for the difference in prevalence of 25(OH)D % <75 nmol/L and % <50 nmol/L determined by mixed model logistic regression adjusting for season, parental smoking, breastfeeding, and time × breastfeeding. *Different from the meat group, P < 0.05.

1), whereas the seasonal variation in the study milk groups was dramatically attenuated. Frequencies of certain seasons within some study groups were too small to examine season-specific effects of the interventions.

The relation between serum 25(OH)D concentrations and the mean vitamin D intake consumed from the vitamin D-fortified study milks showed a linear response (Fig. 2). Post hoc dose–response analysis in the group of toddlers consuming study milk (both milk groups combined) indicated a significant dose-by-season interaction on 25(OH)D concentrations (Table 4). No associations were found between the amount of vitamin D consumed and the concentration of 25(OH)D in the summer (P = 0.73) or fall (P = 0.23); however, there was strong evidence of a serum 25(OH)D response to the amount of vitamin D consumed from fortified milk during the winter months (P < 0.001) with a tendency for vitamin D intake to improve 25(OH)D status during the spring (P = 0.052). Specifically, serum 25(OH)D concentrations in the winter months were 9% higher for every 1 μg of vitamin D consumed.

Discussion

In New Zealand (latitude ranging from 35°S to 47°S), very few foods are fortified with vitamin D, and there are no public health recommendations issued to promote supplementation of vitamin D to infants and children. The Toddler Food Study herein provided the opportunity to evaluate changes in serum 25(OH)D status and PTH concentration in response to a moderate level of vitamin D intake delivered to young, healthy children via fortified milks. Our findings indicate that habitual consumption of vitamin D-fortified milk, providing a mean intake of nearly 4 μg/d during the 20-wk intervention period, was effective in optimizing year-round serum 25(OH)D concentrations (≥50 nmol/L) for most children in this group. The relative contribution of vitamin D intake from the fortified study milks was most effective during the winter when dermal production of vitamin D is markedly diminished. However, despite the beneficial effect on vitamin D status, there was no evidence of a difference in PTH concentrations among the 3 treatment groups.

The present study is the first controlled trial to our knowledge to show an effect of vitamin D intake on circulating 25(OH)D concentrations in this young age group. Evidence on which to base the DRI for toddlers is often inadequate and, in the case of vitamin D, has been derived by extrapolating data from studies of older children and adults (1). The establishment of separate recommended vitamin D intakes for children aged 1–3 y compared to 4–8 y old was based biologically on the more rapid growth rate of toddlers compared to older preadolescent children (1). In the present study, fortification of study milks with vitamin D at a mean level of 0.9 μg/100 g prepared drink provided assigned participants with a mean intake of nearly 4.0 μg vitamin D/d (148 IU/d). This amount is more than 60% lower than the recently updated EAR of 10 μg/d (400 IU/d) set for this age group by the Institute of Medicine on the basis of the selected target serum 25(OH)D concentration of 40 nmol/L (1). Nonetheless, the season-adjusted mean 25(OH)D concentrations of 70 nmol/L among study participants consuming vitamin D-fortified study milks were similar to the blood concentrations reported in young children residing in the United States (3) and were well above the 40- and 50-nmol/L cutpoints. It should be noted that the Institute of Medicine’s Committee estimated the required vitamin D under conditions of minimal sun exposure, which may not be fully met at latitudes below 49°N. The Committee also selected an estimated intake that was anticipated to “overshoot” the targeted serum 25(OH)D concentrations in an effort to err on the side of caution due to uncertainties in the simulated dose–response relationship (1).

The relation of the response of serum 25(OH)D to vitamin D intake in the present study suggests a linear increase in serum 25(OH)D concentration within this moderate dose range. In the winter months, an intake of 1 μg vitamin D/d was associated with ~9% higher concentration of serum 25(OH)D. The Institute of Medicine Committee’s report suggests a nonlinear response of serum 25(OH)D concentrations to doses of vitamin D (1). Specifically, the Committee found a steeper rise in serum 25(OH)D concentrations when vitamin D dosing was <25 μg/d, with an

FIGURE 1  Serum 25(OH)D concentrations by month beginning in March (end of summer) in 177 toddlers during a 20-wk intervention with red meat, vitamin D-fortified milk, or micronutrient-fortified milk. Values are geometric mean (95% CI). For March to December, respectively: n = 2, 5, 4, 1, 7, 10, 8, 10, 11, and 16 (meat); n = 3, 3, 4, 2, 5, 10, 7, 12, 11, and 15 (vitamin D-fortified milk); and n = 1, 2, 1, 4, 3, 5, 6, and 9 (micronutrient-fortified milk). 25(OH)D, 25-hydroxyvitamin D.

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of 11 ng/L) among our study participants compared to reported concentrations ranging from 22 to 42 ng/L (31,36–38). The suppression of PTH to reduce bone resorption in adults is deemed by some a reasonable goal, yet it is unknown whether a decrease in serum PTH concentrations in young children would have beneficial or detrimental effects on skeletal growth. Furthermore, given the strong interdependence of vitamin D and calcium, the relatively high dietary calcium intakes of our study population as a whole may have been sufficient to suppress the rise in serum PTH concentrations (39–41). Previous supplementation trials in adolescent females with moderate- to high-calcium intakes have also reported a lack of change in serum PTH despite supplementation up to 25 μg/d vitamin D (42,43).

Measurement of bone biomarkers and bone mass and density assessments in the present study may have contributed to a better understanding of the association of vitamin D and PTH with bone health in this young age group.

There are several limitations to our study, notably the relatively low habitual intake of vitamin D provided by the study milks rather than a graded dose–response design (approaching intake concentrations nearer to the current recommended allowance of 10–15 μg/d), which would have increased our confidence in the estimate of the intake–response relation. We also did not measure “background” dietary vitamin D intakes from food sources other than the study milk; however, the absence of widespread fortification of foods with vitamin D in New Zealand makes it unlikely that the results were confounded by vitamin D intake from other fortified food sources. Furthermore, foods naturally rich in vitamin D are likely not to be consumed on a regular basis in this life stage group (44). Lastly, a measurement of sun exposure to assess the relative contribution of UVB radiation to the overall serum 25(OH)D concentrations was not collected. The seasonal decline in serum 25(OH)D concentrations in the meat group (control arm in the current study) does suggest negligible UVB exposure from late fall to early spring. Thus, the attenuation of the seasonal effect on serum 25(OH)D concentrations in the treatment groups can be reasonably associated with intake of vitamin D from the study milks.

In summary, the findings of our study highlight the role of food fortification in achieving year-round vitamin D adequacy in toddlers. Using serum 25(OH)D as a biomarker of exposure, consumption of milk fortified with vitamin D at a concentration of 0.9 μg (36 IU) cholecalciferol/0.1 L appears to ensure that the majority of toddlers residing at a latitude of 46°S meet the targeted serum 25(OH)D concentration of at least 50 nmol/L. Although further research is needed to investigate the interre-

### TABLE 4

<table>
<thead>
<tr>
<th>Dose effect by season</th>
<th>Ratio of geometric means (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>1.01 (0.93, 1.10)</td>
</tr>
<tr>
<td>Fall</td>
<td>1.03 (0.98, 1.07)</td>
</tr>
<tr>
<td>Winter</td>
<td>1.09 (1.04, 1.14)</td>
</tr>
<tr>
<td>Spring</td>
<td>1.03 (1.00, 1.08)</td>
</tr>
</tbody>
</table>

1 Values were derived from mixed model adjusted for parental sex, education, and smoking (baseline, n = 101; 20 wk, n = 98) and represent the ratio of geometric mean values (95% CI), which reflect differences in geometric mean serum 25(OH)D concentrations for every 1-μg increase in vitamin D intake (i.e. 1.09 means a 9% increase/1 μg). The dose × season interaction was significant, \( P = 0.033 \). 25(OH)D, 25-hydroxyvitamin D.
relationship of calcium intake, vitamin D status, and bone outcomes, our results underscore the limitation of relying on PTH to assess the impact of vitamin D supplementation in children. Vitamin D-fortified foods studied designed to elucidate the dose–response relationship are also needed to fully address the efficacy of fortification on vitamin D status.

Acknowledgments

We thank Karl Bailey and Michelle Harper for carrying out the laboratory assays. E.A.S.-G., E.L.F., and A-L.M.H. designed research; E.A.S.-G. conducted research; A.R.G. and L.A.H. preformed the data analysis; and L.A.H. wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


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