ABSTRACT

Background: Despite increased awareness of the adverse health effects of low vitamin D status, few studies have evaluated 25-hydroxyvitamin D [25(OH)D] status in young children.

Objectives: We aimed to assess vitamin D status on the basis of 25(OH)D and its relation with parathyroid hormone (PTH) and to identify possible predictors of 25(OH)D status in young children living in a country with minimal vitamin D fortification.

Design: Serum 25(OH)D and PTH concentrations were measured in a cross-sectional sample of children aged 12–22 mo [n = 193 for 25(OH)D, n = 144 for PTH] living in Dunedin, New Zealand (latitude: 45°S). Anthropometric, dietary, and sociodemographic data were collected.

Results: The majority of children sampled in the summer (94%; 47 of 50) had 25(OH)D >50 nmol/L; however, nearly 80% of children sampled in the winter (43 of 55) had serum concentrations ≤50 nmol/L. In season-adjusted multivariate analysis, breastfeeding and higher levels of education were independently associated with lower 25(OH)D concentrations, whereas male sex and cigarette-smoke exposure were positively associated with 25(OH)D (all P < 0.05). Fractional polynomial regression was used to describe the nonlinear relation between serum PTH and 25(OH)D (P < 0.001). When 25(OH)D concentrations were >60–65 nmol/L, a plateau in PTH was evident.

Conclusions: Seasonal variation in 25(OH)D concentration implies that postsummer vitamin D stores were insufficient to maintain status >50 nmol/L year-round. Examination of the predictors of 25(OH)D in our model shows few modifiable risk factors, and thus effective dietary strategies may be required if future research determines that children with 25(OH)D concentrations ≤50 nmol/L are at significant health risk. This trial was registered at www.actr.org.au as ACTRN12605000487617. Am J Clin Nutr 2010;92:69–76.

INTRODUCTION

Vitamin D deficiency is associated with impaired intestinal calcium absorption resulting in compensatory hyperparathyroidism, increased bone resorption, and decreased bone integrity. Serum 25-hydroxyvitamin D [25(OH)D] concentrations serve as the best available marker for vitamin D status. Whereas a severe deficit in 25(OH)D concentrations in children leads to rickets and growth failure (1), an increasing body of evidence suggests that depletion of bone mineral also occurs with less severe degrees of vitamin D deficiency (2–7). This is speculated to contribute to increased fracture risk and the development of osteoporosis later in life. Moreover, results from observational studies suggest that vitamin D supplements in infancy and early childhood may decrease the incidence of type 1 diabetes (8), whereas epidemiologic studies over the past 2 decades have suggested important effects of vitamin D on the immune system and in prevention of cardiovascular disease and certain cancers (9).

Despite the high level of interest in the possible adverse health effects of low vitamin D status, little is known about serum 25(OH)D concentration and its predictors in young children. In the United States, serum 25(OH)D concentrations were measured for children aged 1–5 y in the 2 most recent cycles of the National Health and Nutrition Examination Survey (NHANES 2003–2004 and 2005–2006). Approximately 14% of children in this age group were reported to have 25(OH)D concentrations ≤50 nmol/L, although it is likely this prevalence was an underestimate as samples were collected during the summer at higher latitudes (further north) and during the winter at lower latitudes (further south) (10). In contrast, a higher prevalence of low vitamin D status [25(OH)D < 50 nmol/L] of nearly 20% was found in a nationally representative sample of children of the United Kingdom aged 1.5–4.5 y when surveyed throughout the year (11). These high prevalence rates of suboptimal vitamin D status occurred despite widespread fortification of vitamin D in margarines, milk, breakfast cereals, and juices in both the United States and the United Kingdom. To date, few studies have assessed year-round vitamin D status in young children in countries where few foods in the national food supply are fortified.

Although the best indicator of vitamin D status is serum 25(OH)D concentration, additional information on the functional role of vitamin D in bone health can be obtained by evaluating the 25(OH)D threshold required for maximal suppression of parathyroid hormone (PTH) concentration. Studies assessing the...
association of serum 25(OH)D and PTH concentrations have been largely based on older children and adults (4, 5, 12–19). Little information is available about whether a plateau in PTH concentration is also evident in young children.

Furthermore, examination of seasonal, demographic, and lifestyle influences on vitamin D status is essential for the development of population-specific public health recommendations, particularly in countries such as New Zealand where fortification is neither mandated nor common. Thus, the aims of this study were to assess vitamin D status on the basis of serum 25(OH)D concentration and its relation to PTH and to identify possible predictors of 25(OH)D in a group of young children aged 12–22 mo residing in Dunedin, New Zealand (latitude: 45°S).

SUBJECTS AND METHODS

Study participants

This study used baseline data from the Toddler Food Study (20), an intervention trial that investigated the effect of providing red meat or iron- and zinc-fortified milk on the micronutrient status of New Zealand children aged 12–22 mo residing in Dunedin and the surrounding areas (latitude: 45°S). This site has a temperate climate with a summer mean temperature of 14°C and a winter mean temperature of 5°C (21). Two-hundred twenty-five children were recruited between February 2004 and August 2005. For the current project, children who were missing serum 25(OH)D (n = 32) and PTH (n = 49 of those with serum 25(OH)D data at baseline were excluded, leaving 193 and 144 participants for analysis, respectively. Approval to conduct the study 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.

Biochemical measurements

Nonfasting blood samples for 25(OH)D measurements were obtained by venipuncture into a Vacutainer (Becton Dickinson, Franklin Lakes, NJ) with no anticoagulant, processed immediately, and stored at −80°C. Serum 25(OH)D was measured with a radioimmunoassay kit (Diasorin, Stillwater, MN) with an analytic sensitivity of 4 nmol/L. The control samples provided by the manufacturer were within the recommended range, and the interassay CV based on a pooled serum was 13% (n = 5). An electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, Tenzberg, Germany) was used for PTH measurement. The PTH assay showed a detection sensitivity of 1.2 pg/mL, and its intra- and interassay variations were <6% (CV 6% for 46 pg/mL).

Dietary assessment

Calcium intakes were assessed by 3-d weighed diet records collected over 2 wk on randomly selected nonconsecutive days, consisting of 2 weekdays and a weekend day, and analyzed with the use of computer software (Diet Cruncher for Macintosh version 1.2.0; Ross Marshall-Seeley, Way Down South Software, Dunedin, New Zealand) incorporating the New Zealand Food Composition database (22). Dietary vitamin D intakes were not assessed because the New Zealand Food Composition Database has incomplete vitamin D data. However, few foods are fortified with vitamin D in New Zealand, and foods naturally rich in vitamin D, such as fatty fish and liver, are not frequently consumed by young children in this population.

Anthropometric measurements and other covariates

Measurements of weight and length were taken according to standardized procedures (23) and measured to the nearest 0.1 kg and 0.1 cm, respectively. Each child’s nude weight was measured to the nearest 0.1 kg with digital scales (Seca 770 Alpha; Seca Corp, Hamburg, Germany), and recumbent length was measured with a calibrated pediatric length board (O’Leary; Ellard Instrumentation Ltd, Seattle, WA). Body mass index (BMI; in kg/m²) was calculated. Anthropometric measurements were expressed as age- and sex-specific SDs from the mean (z scores) on the basis of World Health Organization (WHO) Child Growth Standards (24).

Sociodemographic (including age, sex, skin tone, ethnicity, and caregiver educational level) and health behavior (including dietary intake, exposure to cigarette smoke in the household, and breastfeeding status) data were collected from the consenting caregiver with a self-administered questionnaire. Participant skin tone was assessed by the primary caregiver, whereby the caregiver matched skin, which was not exposed to the sun, to 1 of 7 color swatches classifying skin tone as either very fair, fair, medium, olive, dark, very dark, or black. There were no participants reported to have very dark or black skin tone. Skin tone was then collapsed into 2 categories: very fair/fair and medium/olive/dark. Education was assessed on the highest qualification of the primary caregiver and classified into 2 groups: postsecondary education (university or college) or secondary school or less. Exposure of the toddler to cigarette smoke was defined by the number of days per week that the toddler was in the same room as someone who was smoking. Cigarette smoke exposure was then classified into 2 groups: never or one of more days per week. Participant ethnicity was classified by caregiver identification of the child as Maori, Pacific, or New Zealand European and others with free-text descriptions provided for the last category. The others included participants further identified as other European (non-New Zealand European; n = 6), Asian (n = 6), Mediterranean (n = 1), and Middle Eastern (n = 1). Ethnicities were then collapsed into Maori/Pacific/Asian/Mediterranean/Middle Eastern (representing generally darker skin pigmentation) and New Zealand European/Other European (representing generally lighter skin pigmentation), with the former being prioritized over the latter where both were reported by the caregiver.

Statistical analysis

All analyses were performed with Stata (version 10.1; Stata Corp, College Station, TX). Serum 25(OH)D concentration was log transformed before statistical analysis where this resolved issues with skew and/or heteroscedasticity in the residuals from regression models with 25(OH)D as an outcome. All tests were 2-sided with statistical significance determined by P < 0.05.

To examine factors related to serum 25(OH)D, the following known or potential predictors were identified: age, sex, BMI z score (age- and sex-standardized), prioritized ethnicity, skin tone, educational level of primary caregiver, exposure of child to...
cigarette smoke, and whether the child was currently breastfeeding. Regression models examining each of these potential predictors were constructed and included season in each case as this was expected to explain a large proportion of the variation in serum 25(OH)D concentrations. Season was coded in 4 categories on the basis of the astronomical solstice and equinox dates: summer, 21 December to 20 March; autumn, 21 March to 20 June; winter, 21 June to 21 September; and spring, 22 September to 20 December. Those variables with $P < 0.20$ in their model or that had been a priori selected (namely season, age, sex, and breastfeeding) were then included in the final multiple regression model to examine the independent contribution of these potential predictors. Nonlinearities in the associations between serum 25(OH)D and continuous predictors (age and BMI $z$ scores) were investigated with the use of fractional polynomials. Interactions between sex and age, season and sex, season and breastfeeding, and season and age were investigated in the multivariate regression model. The relation between serum 25(OH)D and PTH concentrations was evaluated with fractional polynomial regression (25) controlled for dietary calcium intake. Bootstrapping was used to obtain CIs for the point of maximal suppression on the basis of 1000 bootstrapped samples and defining maximal suppression as the lowest concentration of 25(OH)D where PTH is = 25% higher than its lowest (positive) value. This is approximately equivalent to the approach where $-3C$ is the estimated point of maximal suppression (17) and an exponential model $[y = A + B \times e^{-C/25(OH)D}]$ was applied to this data set with an additional linear term for dietary calcium (the approximate estimates being $A = 10, B = 50$, and $C = -0.065$). A 95% CI was obtained from the percentile method.

RESULTS

Subject demographics and growth characteristics

The median age of the 193 children with serum 25(OH)D measurements was 17 mo (age range: 12–22 mo), and the majority of children were New Zealand European and Other Europeans (84%). The median height-for-age $z$ score of 0.1 (25th and 75th percentiles: −0.6, 0.9) indicated typical linear growth status, whereas the $z$ score for BMI of 0.8 (25th and 75th percentiles: 0.2, 1.4) suggested that the children were heavier than the WHO Child Growth Standards (24). Of these 193 children, 37 (19%) were still breastfeeding and 7 (4%) were consuming milk formula at the time of enrollment. None of the children were receiving supplemental vitamin D. Median dietary calcium intakes (25th and 75th percentiles) were 762 mg/d (579, 921 mg/d) (one missing value) with the average intake of calcium (mean ± SD: 771 ± 280 mg/d) exceeding the Adequate Intake of 500 mg (26).

Distribution and evaluation of 25(OH)D concentrations

Serum 25(OH)D concentrations ranged from 14.5 to 167.8 nmol/L. The monthly variation in serum 25(OH)D concentrations is shown in Figure 1. The variability during the summer months was greater than during the winter months. Compared with the month of January (summer), concentrations of 25(OH)D were appreciably lower from April (autumn) through October (early spring). The nadir in ultraviolet (UV) radiation occurs midwinter (July) after the peak 6 mo earlier in summer (December). Monthly mean 25(OH)D concentration closely mirrors the average monthly predicted UV index (expected at solar noon on a cloudless day) (27). UVB wavelengths are present when the UV index is > 3.

Because of the marked seasonal variation, a detailed stratification of season-adjusted serum 25(OH)D concentrations according to demographic characteristics is presented in Table 1. Neither age nor BMI $z$ score was transformed because linear associations were adequate in both cases. These results should be interpreted with caution due to the possibility of confounding. The overall season-adjusted geometric mean 25(OH)D (95% CI) concentration of participants was 52.3 nmol/L (49.1, 55.8 nmol/L). Boys had a statistically significant higher mean year-round serum 25(OH)D concentration [15% higher (95% CI: 3%, 28%)] than did girls ($P = 0.016$), with adjustment for season. There was no statistically significant effect of ethnicity or skin tone on serum 25(OH)D concentrations.

The percentage of participants with 25(OH)D concentrations < 27.5 and < 50 nmol/L is shown by season in Figure 2. In the summer, the majority of children had serum 25(OH)D concentrations ≥ 50 nmol/L, and none of the children presented with 25(OH)D concentrations < 27.5 nmol/L. In contrast, only 12 of 55 (22%) children sampled in the winter exhibited serum concentrations ≥ 50 nmol/L, with 9 (16%) of the children having concentrations < 27.5 nmol/L.

Relation between 25(OH)D and PTH concentrations

Serum PTH concentrations were available for 144 participants and are reported based on medians and percentiles due to non–log normal skew. The median concentration of serum PTH (25th and 75th percentile) was 8.4 pg/mL (3.1, 15.4 pg/mL). Fractional polynomial regression controlled for dietary calcium intake showed that there was a statistically significant association between serum 25(OH)D and PTH with 11.3% of variation

FIGURE 1. Geometric mean (and 95% CI) serum 25-hydroxyvitamin D [25(OH)D] concentrations by month in 193 young children (aged 12–22 mo) and the average monthly predicted ultraviolet (UV) index (expected at solar noon on a cloudless day) in Dunedin, New Zealand (latitude: 45°S).
explained by the model \((P < 0.001)\) and with a marked increase in serum PTH at serum 25(OH)D concentrations \(60–65 \text{ nmol/L}\). (Figure 3).

Serum 25(OH)D was transformed as the reciprocal of the square of the original value. This plateau persisted even with the 4 largest values of 25(OH)D removed. The point of maximal suppression on this data set was 61.4 (95% CI: 42.0, 167.8). The width of this CI shows the high degree of uncertainty about the point of maximal suppression and caution should be applied in interpreting this estimate. This model found no evidence of an association between dietary calcium and PTH \((P = 0.248)\).

Selected predictors associated with serum 25(OH)D concentrations

Regression analyses of the association between predictors and serum 25(OH)D are presented in Table 2. Age was not transformed because a linear association was adequate. From a univariate regression model, season at time of blood collection explained the greatest proportion of variation in log serum 25(OH)D (29.3%, \(P < 0.001\)). In multivariate regression models adjusted for season, sex \((P = 0.031)\), current breastfeeding \((P = 0.032)\), educational level attained by primary caregiver \((P = 0.040)\), and exposure of child to cigarette smoke \((P = 0.044)\) were independently related to 25(OH)D concentrations, but age was not \((P = 0.596)\). Specifically, the geometric mean concentration of 25(OH)D was 14% lower (95% CI: 1%, 25%) in children still breastfeeding and 11% lower (95% CI: 1%, 21%) in children whose caregivers had a higher level of education. In contrast, geometric mean 25(OH)D was 13% higher (95% CI: 1%, 26%) in boys and 33% higher (95% CI: 1%, 76%) in children exposed to cigarette smoke. Effects of collinearity were checked for in this model and found to be minimal with very low variance inflation factors of 1.11 or lower for age, sex, breastfeeding, education, and exposure to cigarette smoke. Linear combinations of these variables explained an additional 7.0% of the variation in log serum 25(OH)D concentrations of children beyond that explained by season, for a total of 36.3% of variation explained in the final multivariate model. The additional variation explained by each variable by squared semipartial correlations was similar for sex (1.7%), breastfeeding (1.7%), education (1.6%), smoking (1.5%), and age (0.1%). None of the interactions investigated were statistically significant: age and sex \((P = 0.999)\), season and sex \((P = 0.073)\), season and breastfeeding \((P = 0.601)\), and season and age \((P = 0.108)\).

**DISCUSSION**

The mean 25(OH)D concentration of 52 nmol/L in our young 12–22-mo-old children in the present study was similar to that

### TABLE 1

<table>
<thead>
<tr>
<th>Participants</th>
<th>Mean serum 25(OH)D (95% CI)</th>
<th>Change (95% CI)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Season at time of blood collection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>50 (26)</td>
<td>74.1 (66.5, 82.5)</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>60 (31)</td>
<td>49.2 (44.6, 54.3)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Winter</td>
<td>55 (29)</td>
<td>38.7 (34.9, 42.8)</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>28 (15)</td>
<td>58.0 (50.3, 67.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>82 (42)</td>
<td>48.7 (44.8, 52.8)</td>
<td>(0.016)</td>
</tr>
<tr>
<td>Male</td>
<td>111 (58)</td>
<td>55.2 (51.4, 59.3)</td>
<td>(0.342)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand European and other European</td>
<td>163 (84)</td>
<td>53.4 (50.3, 56.7)</td>
<td>(0.855)</td>
</tr>
<tr>
<td>Maori, Pacific Islander, Asian, Mediterranean, and Middle Eastern</td>
<td>30 (16)</td>
<td>46.9 (40.8, 53.9)</td>
<td>(0.108)</td>
</tr>
<tr>
<td><strong>Skin tone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very fair and fair</td>
<td>105 (56)</td>
<td>52.0 (48.3, 56.0)</td>
<td>(0.022)</td>
</tr>
<tr>
<td>Medium, olive, and dark</td>
<td>84 (44)</td>
<td>52.6 (48.4, 57.2)</td>
<td>(0.050)</td>
</tr>
<tr>
<td><strong>Education of primary caregiver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postsecondary education</td>
<td>117 (64)</td>
<td>51.2 (47.8, 54.8)</td>
<td>(0.008)</td>
</tr>
<tr>
<td>Secondary school or less</td>
<td>65 (36)</td>
<td>54.2 (49.5, 59.3)</td>
<td>(0.094)</td>
</tr>
<tr>
<td><strong>Exposure to cigarette smoke</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (6)</td>
<td>66.8 (53.2, 83.9)</td>
<td>(0.022)</td>
</tr>
<tr>
<td>No</td>
<td>182 (94)</td>
<td>51.6 (48.7, 54.5)</td>
<td>(0.073)</td>
</tr>
<tr>
<td><strong>Currently breastfeeding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37 (19)</td>
<td>47.5 (41.9, 53.7)</td>
<td>(0.683)</td>
</tr>
<tr>
<td>No</td>
<td>156 (81)</td>
<td>53.5 (50.4, 56.9)</td>
<td>(0.999)</td>
</tr>
<tr>
<td>Age (per mo)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI z score (per unit)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values were derived from linear regression models adjusted for season.
2 Seasons were defined as follows: autumn, 21 March to 20 June; winter, 21 June to 20 September; spring, 22 September to 20 December; and summer, 21 December to 20 March.
3 Exposure to cigarette smoke in the household on \(\geq 1\) d/wk.
observed in the 2002 National Survey of New Zealand children aged 5–14 y (mean concentration of 50 nmol/L) (28, 29). Season at the time of blood collection explained a substantial proportion of the variance in 25(OH)D characterized by a 35 nmol/L decline from summer to winter. A substantial proportion (16%) of participants assessed in the winter months had 25(OH)D concentrations <27.5 nmol/L, a concentration associated with increased risk of clinical myopathy, rickets, and osteomalacia (30). However, defining an absolute threshold concentration for 25(OH)D for vitamin D sufficiency and deficiency states in children is difficult due to the paucity of functional outcome data associated with any 25(OH)D concentration. The American Academy of Pediatrics has recently recommended that serum 25(OH)D concentrations in infants and children should be maintained at >50 nmol/L (31), the concentration attributed to vitamin D sufficiency in adults. With this cutoff, we found that nearly 80% of children sampled in the winter had 25(OH)D concentrations <50 nmol/L. More studies examining the associations of calcium absorption and measurements of bone-related health outcomes with 25(OH)D concentrations in children are needed to determine whether levels for sufficiency being used in adults can be appropriately applied to children. In the absence of experimental and observational data, the use of this cutoff as a target of vitamin D adequacy in this age group should be interpreted with caution.

In adults, studies suggest that vitamin D sufficiency should be categorized on the basis of physiologic response of PTH concentrations to various serum 25(OH)D concentrations. Although there is evidence for an inverse relation between 25(OH)D and serum PTH in older children and adolescents, estimates of 25(OH)D required for maximal suppression of PTH vary from 30 to 100 nmol/L (4, 12, 13, 15, 18, 32). In our study, a serum 25(OH)D concentration of ≥61 nmol/L was needed to minimize PTH concentrations. Most (82%) of the subjects had 25(OH)D concentrations below this value; however, there are concerns regarding the interpretability of PTH concentrations in defining 25(OH)D cutoffs in children. To our knowledge, there are no studies in this age group that have investigated whether the threshold of serum 25(OH)D concentrations associated with maximal suppression of PTH differentiates between desirable and adverse effects on bone health. Moreover, normal values for PTH concentration in actively growing children have not been defined, in part because the range of PTH concentrations that correlates with normal bone turnover in children has yet to be determined (33).

Latitude is a well-established risk factor for hypovitaminosis D, particularly in countries with minimal fortification practices (34). Geometric mean 25(OH)D concentration (38.7 nmol/L) in children sampled in the winter in Dunedin (45°S) were nearly 10 nmol/L lower than the median for a group of infants and young children, also sampled in the winter but residing at a higher latitude in Auckland, New Zealand (34°S) (35). Measurement of UV radiation in New Zealand reveals that, on average, Leigh, New Zealand, which is at 36.5°S (a similar latitude to Auckland), receives 10% more UV radiation in the summer.

![FIGURE 2. Seasonal prevalence of low serum 25-hydroxyvitamin D concentrations defined as <27.5 nmol/L or 27.5–50 nmol/L in 193 young children (aged 12–22 mo).](https://academic.oup.com/ajcn/article-abstract/92/1/69/4597421)

![FIGURE 3. Serum parathyroid hormone (PTH) by 25-hydroxyvitamin D [25(OH)D] concentration in 144 young children (aged 12–22 mo). The slope of the fractional polynomial regression line controlled for dietary calcium intake is shown. $\text{PTH}_{\text{ng/ml}} = 11 + 5900 \times (25\text{(OH)D}_{\text{nmol/L}})^2 - 0.00028 \times \text{Camg}$.](https://academic.oup.com/ajcn/article-abstract/92/1/69/4597421)

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Independent factors predicting serum 25-hydroxyvitamin D concentrations in young children aged 12–22 mo$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explanatory variables</td>
<td>Ratio of means</td>
</tr>
<tr>
<td>Season at blood collection$^a$</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>0.70</td>
</tr>
<tr>
<td>Winter</td>
<td>0.51</td>
</tr>
<tr>
<td>Spring</td>
<td>0.78</td>
</tr>
<tr>
<td>Sex</td>
<td>1.13</td>
</tr>
<tr>
<td>Education of primary caregiver</td>
<td>0.89</td>
</tr>
<tr>
<td>Exposure to cigarette smoke</td>
<td>1.33</td>
</tr>
<tr>
<td>Age (per mo)</td>
<td>0.99</td>
</tr>
<tr>
<td>Currently breastfeeding</td>
<td>0.86</td>
</tr>
</tbody>
</table>

$^a$ Values were derived from a multiple linear regression model and represent the ratio of geometric mean values, which reflect mean differences in serum 25-hydroxyvitamin D concentrations (nmol/L) in blood collected in the autumn, winter, or spring compared with summer; for boys compared with girls; for postsecondary education (university or college) attained by primary caregiver compared with secondary school or less; for exposure to cigarette smoke on ≥1 d/wk compared with not having been exposed to cigarette smoke; and for currently breastfeeding (any) compared with not currently breastfeeding.

$^2$ Seasons were defined as follows: autumn, 21 March to 20 June; winter, 21 June to 21 September; spring, 22 September to 20 December; and summer, 21 December to 20 March.
and twice as much in the winter than Lauder, New Zealand, which is at 45.0°S (the same latitude as Dunedin) (36). In countries where the addition of vitamin D to foods is both substantial and widespread, the latitudinal increase in the length of the vitamin D winter is attenuated. For example, the seasonal decline in 25(OH)D concentrations in a group of healthy young children residing in Boston, MA (42°N), a similar latitude to our New Zealand locale, was much less pronounced than that described in our study participants (7). In fact, the authors in the US study observed minimal seasonal variation in 25(OH)D status, with 25(OH)D concentrations <50 nmol/L ranging from 18% of participants in summer to only 7% in winter (7). Not surprisingly, an inverse relation between milk consumption and the risk of participants presenting with 25(OH)D concentrations <50 nmol/L was shown in these US children, thereby supporting the efficacy of the vitamin D fortification practices implemented in the United States.

To further explore the determinants of vitamin D status in this age group, we investigated other potential predictors of serum 25(OH)D status. Children who were breastfeeding had on average an ≈14% lower circulating concentration of 25(OH)D. Although vitamin D deficiency is common among breastfed infants who do not receive supplemental vitamin D (7, 37, 38), this is the first study to show lower vitamin D status among children breastfed beyond the first year of life. Whereas only 37 of 193 participants (19%) were currently breastfeeding at the time of blood collection, the majority of children (140 of 193; 92%) were reported to have breastfed at some point before study enrollment. Rates of breastfeeding among Dunedin mothers are high, with 88% initiating breastfeeding, 42% exclusively breastfeeding at 3 mo, and 34% partially breastfeeding at 12 mo (39). Many mothers are vitamin D–insufficient during pregnancy (40–44); thus, infants are born with suboptimal stores. Breast milk generally does not contain sufficient vitamin D to meet the needs of infants (45), and there are no public health initiatives in New Zealand for vitamin D supplementation in infants who are exclusively breastfed. Moreover, it is not common practice to intentionally expose infants to sunlight. Instead “sun safe” messages to avoid sun exposure are widely and strongly promoted because of concern about the high rates of melanoma in New Zealand.

We also observed higher 25(OH)D concentrations in boys and in children exposed to cigarette smoke. Although the reason for the apparent sex difference is unclear, several studies have reported higher vitamin D status in boys compared with girls among older children (28, 46, 47). It has been suggested that the higher serum concentrations could be because of increased sunlight exposure in boys through the association with higher levels of physical activity (28). We did not measure sun exposure or physical activity in this study. However, it seems highly unlikely that sex differences in physical activity, in particular outdoor activity, exist within this young age group. The higher 25(OH)D concentration seen among children exposed to cigarette smoke disagrees with the findings of several adult studies, which have found smoking to be associated with a low bone mass and reduced concentrations of serum 25(OH)D and PTH (48, 49). Cigarette smoking is often accompanied by multiple high-risk health behaviors, including increased unprotected sun exposure (50). Although the literature is scant, studies have found that parental sun protection behavior is a strong predictor of child sun protection practices (51, 52). Thus, it is possible that exposure of the child to cigarette smoke may be indirectly associated with their lower use of sun protection and consequently greater UVB exposure. Cigarette smoke exposure was collected by parental report, and may be subject to misclassification (53). For this reason, the reported association should be interpreted with caution.

The negative association of children’s 25(OH)D concentration with a higher caregiver educational level has not been well described. A possible explanation could be that caregivers with a higher educational level may be more likely to apply sunscreen and dress the child in protective clothing. Indeed, a survey designed to explore the use of sun protection behaviors in New Zealand children indicated that the level of parental education was one of the potential predictors of parental intention to let their child get a suntan (54).

Last, 25(OH)D concentration was not found to vary with age or by BMI z score. Prior studies have reported an inverse association of vitamin D status with age during childhood and adolescence (32, 47, 55, 56). Our analysis, however, was limited due to a narrow age range. Obesity in children has also been associated with lower 25(OH)D concentrations (7, 28, 47, 56–58), although not all studies have found an association between fat mass and vitamin D status (32, 46, 59). This association may be because of both the sequestration of vitamin D in fat tissue and the possibly more sedentary, indoor lifestyle of obese children. Although the z scores in the present study suggested that BMI was higher in our population than the WHO Child Growth Standards (24), very few children were obese (2%, n = 4). Likewise, a relation between weight status and indoor lifestyle may be more pronounced in older than in younger children.

In conclusion, the large seasonal variation in 25(OH)D concentrations presented herein highlights the cyclic course of year-round vitamin D status in young children residing in the southern latitudes of New Zealand. The clinical significance of these changes is unknown. Few modifiable risk factors for preventing these marked seasonal variations seen in this population were identified in our model; however, vitamin D intake and sunlight exposure, modifiable factors known to contribute to vitamin D status, were not evaluated. In the absence of widespread fortification of foods with vitamin D in New Zealand, it is likely that vitamin D intakes were well below the recommended intake of 5.0 µg/d (60). Although sunlight exposure is potentially modifiable, sun safe messages to avoid sun exposure are very strongly promoted in New Zealand and are unlikely to be altered because of the high risk of skin cancer associated with sun exposure. Moreover, the lack of adequate UVB radiation during the winter months would make advice to modify sun exposure ineffective for part of the year. Should the vitamin D status of our participants be found to be suboptimal for child health, it will be crucial to determine effective dietary strategies to increase the vitamin D intake of New Zealand children.

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VITAMIN D STATUS OF YOUNG NEW ZEALAND CHILDREN


