Modifiable Determinants of Serum 25-Hydroxyvitamin D Status in Early Childhood

Opportunities for Prevention

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Objectives: To determine the effect of modifiable dietary intake variables (current vitamin D supplementation and daily cow’s milk intake) on 25-hydroxyvitamin D level in early childhood and to evaluate the relationship between these modifiable dietary factors and other largely nonmodifiable determinants of vitamin D status including skin pigmentation and season.

Design: Cross-sectional study.

Setting: Primary care pediatric and family medicine practices participating in the TARGet Kids! practice-based research network in Toronto, Ontario, Canada.

Participants: From December 2008 to June 2011, healthy children 1 to 5 years of age were recruited during a routine physician’s visit.

Interventions: Survey, anthropometric measurements, and laboratory data were collected. A multivariable linear regression model was developed to examine the independent effects of vitamin D supplementation and daily volume of cow’s milk on 25-hydroxyvitamin D level.

Main Outcome Measures: 25-Hydroxyvitamin D level.

Results: Blood was obtained in 1898 children. Two modifiable dietary intake variables, vitamin D supplementation and cow’s milk, increased 25-hydroxyvitamin D level by 3.4 ng/mL (95% CI, 2.4-4 ng/mL) and 1.6 ng/mL per 250-mL cup per day (95% CI, 1.2-2 ng/mL), respectively. Two nonmodifiable variables reflecting cutaneous vitamin D synthesis (skin pigmentation and season) were also strongly associated with 25-hydroxyvitamin D status but accounted for a much smaller proportion of the explained variation in 25-hydroxyvitamin D level. The effect of vitamin D supplementation and milk intake on 25-hydroxyvitamin D level appeared similar regardless of skin pigmentation or season.

Conclusion: Two modifiable dietary intake variables (vitamin D supplementation and cow’s milk intake) are the most important determinants of 25-hydroxyvitamin D status in early childhood.


Yet, the effect of cow’s milk intake and vitamin D supplementation on vitamin D status is unclear, particularly in the context of known nonmodifiable vitamin D determinants such as skin pigmentation and season.

In 2008, the American Academy of Pediatrics Committee on Nutrition recommended that all children older than 1 year receiving less than 1 L of cow’s milk per day be supplemented with 400 IU of vitamin D daily.13 For the first time, healthy children older than 1 year were recommended to receive supplemental vitamin D, providing a unique opportunity to study the effect of vitamin D supplementation and cow’s milk intake on the vitamin D status of young children.

The primary objective of this study was to determine the effect of current vitamin
D supplementation and daily cow’s milk intake on 25-hydroxyvitamin D level in a large cohort of healthy urban preschool children. Our secondary objective was to evaluate the relationship between these modifiable dietary factors and other nonmodifiable determinants of vitamin D status including skin pigmentation and season.

METHODS

SUBJECTS AND DESIGN

In this prospectively designed, cross-sectional observational study, healthy children 1 to 5 years of age were recruited during a routine health maintenance physician’s visit through the TARGet Kids! primary care practice-based research network in Toronto, Ontario, Canada (latitude 43.4°N) between December 2008 and June 2011. TARGet Kids! is a collaboration between University of Toronto child health outcomes researchers and primary care physicians from the Department of Pediatrics and the Department of Family and Community Medicine. Children were excluded if they had any chronic illnesses (excluding asthma), were taking a medication known to alter vitamin D metabolism (ie, phenobarbital), or had a gestational age less than 32 weeks.

SUBJECT RECRUITMENT AND DATA COLLECTION

Study participants were recruited by research personnel embedded in 7 participating pediatric and family medicine practices. Data were collected prospectively through a standardized parent-completed survey instrument based on the Canadian Community Health Survey24; anthropometric measurements including height and weight obtained by trained research assistants; and venous blood sampling collected at the primary care clinic. Medidata RAVE (Medidata Solutions Inc, http://www.mdsol.com/) was used as the secure electronic data capture system and data repository for all TARGet Kids! data.

The primary outcome for this study was 25-hydroxyvitamin D level. Specimens were sent daily to the Clinical Biochemistry Laboratory at Mount Sinai Hospital in Toronto. Total 25-hydroxyvitamin D level was measured from sera samples using a competitive 2-step chemiluminescence assay with a Diasorin LIAISON 25-hydroxyvitamin D TOTAL.28 Extensive testing and validation of this machine has been performed and has demonstrated an intra-assay imprecision of 7.2% at a concentration of 85 ng/mL (to convert to nanomoles per liter, multiply by 2.496) and an interassay imprecision of 4.9% at 13 ng/mL, and 17.4% at 85 ng/mL, values that are well within acceptable limits for biochemical measurements.29,30

The primary predictor variables were vitamin D supplementation and daily volume of cow’s milk intake. These were determined from the response to the question “Does your child take a vitamin D-containing supplement regularly?” and “How many 250-mL cups of cow’s milk does your child drink in a typical day?”

Covariates that might influence vitamin D status were identified through a literature review. Covariates were chosen for inclusion if they were associated with 25-hydroxyvitamin D in 1 or more published studies and included age, sex, season (May–September vs October–April), daily outdoor play time, daily screen time, and skin pigmentation. Weight was measured using a precision digital scale (±0.025%; SECA) and standing height was measured using a stadiometer (SECA). Body mass index was calculated as weight in kilograms divided by height in meters squared.27,31 Body mass index z scores were calculated using World Health Organization growth standards.25 Skin pigmentation was measured by a trained research assistant using the Fitzpatrick scale, which is a 6-category skin pigmentation classification system that has been widely used.7,35

STATISTICAL ANALYSES

Descriptive statistics were performed for the main outcome, predictors, and covariates. For our primary analysis, a multivariable linear regression model was developed to examine the independent effects of vitamin D supplementation and daily volume of cow’s milk on 25-hydroxyvitamin D level with adjustment of prespecified, clinically relevant covariates (described earlier). All covariates were included in the final model irrespective of their associated P values.

To reduce the likelihood of overfitting and obtain more conservative estimates of model performance, bootstrap validation of the linear regression model was performed. Additionally, the bootstrap assessment was repeated 100 times to obtain 95% confidence intervals.34 Missing data were handled by single imputation using conditional means and recursive partitioning.35 A sensitivity analysis including or not including imputed data was also conducted.

For our secondary analyses, we explored the relationship between vitamin D supplementation and milk intake on 25-hydroxyvitamin D level in the context of children’s skin pigmentation and season. To accomplish this, we built on the multivariable linear regression model developed for the primary analyses through the addition of biologically plausible interactions. These included vitamin D supplementation by skin milk intake, vitamin D supplementation by season, vitamin D supplementation by skin pigmentation, and skin pigmentation by season. To achieve a balance between overfitting and interpretation and to limit the biases that can result from standard variable selection approaches, these interactions were tested together. If the joint P value was large (> .30), no further testing was considered. Otherwise, the interactions were retained in the model. To assess the contribution of each variable and interaction on the variation in 25-hydroxyvitamin D level, the partial R² for each was calculated and displayed graphically.

Data were analyzed using the R project for statistical computing.36 This study was approved by the research ethics board at The Hospital for Sick Children, and all parents of participating children consented to participation in the study.

RESULTS

STUDY POPULATION

Of the 3396 children who consented to participate, venous blood sampling was obtained in 1898 children who were included in this analysis (Figure 1). Sev-
enty-six percent of children had complete survey, anthropometric, and laboratory data. Fifty-seven percent of the population were regularly consuming a vitamin D–containing supplement. Mean daily cow’s milk intake was 455 mL. Mean 25-hydroxyvitamin D level was 35 ng/mL (95% CI, 34.86-35.66 ng/mL). Thirty-five percent (95% CI, 33%-38%) had a 25-hydroxyvitamin D level less than 30 ng/mL and 6% (95% CI, 5%-7%) had a 25-hydroxyvitamin D level less than 20 ng/mL (Figure 2). Subject characteristics are presented in Table 1. Imputation for missing values did not change descriptive characteristics.

EFFECT OF VITAMIN D SUPPLEMENTATION AND COW’S MILK INTAKE ON 25-HYDROXYVITAMIN D LEVEL

For our primary analysis, results of the multivariable linear regression model are shown in Table 2. In the adjusted model, the 2 modifiable dietary intake variables, vitamin D supplementation and cow’s milk intake, were highly significant, with vitamin D supplementation increasing 25-hydroxyvitamin D level by 3.4 ng/mL (95% CI, 2.4-4 ng/mL) and cow’s milk increasing 25-hydroxyvitamin D level by 1.6 ng/mL per 250-mL cup per day (95% CI, 1.2-2 ng/mL). Two nonmodifiable variables reflecting cutaneous synthesis were also strongly associated with 25-hydroxyvitamin D level, with summer season increasing 25-hydroxyvitamin D level by 1.6 ng/mL (95% CI, 0.4-2.8 ng/mL) and light skin pigmentation (Fitzpatrick skin pigmentation type I-III vs IV-VI) increasing 25-hydroxyvitamin D level by 2.7 ng/mL (95% CI, 1.2-4.4 ng/mL).

For our secondary analysis, the relationship between modifiable dietary intake variables (vitamin D supplementation and milk intake) on 25-hydroxyvitamin D level was evaluated in the context of children’s skin pigmentation and season. A simultaneous test of all hypothesized interactions resulted in $P = .59$, which is sufficiently large to consider interactions unlikely. The adjusted effects of vitamin D supplementation, milk intake, season, and skin pigmentation on 25-hydroxyvitamin D level are displayed graphically in Figure 3. The effect of vitamin D supplementation and milk intake on 25-hydroxyvitamin D level appear similar regardless of skin pigmentation or season.

To identify the relative contribution of vitamin D supplementation and cow’s milk intake on 25-hydroxyvitamin D level, the partial $R^2$ for each variable in the model was calculated (Figure 4). The 2 modifiable dietary variables, cow’s milk consumption and vitamin D supplementation, appear to account for most of the explained variation in 25-hydroxyvitamin D level. Sensitivity analysis including or not including imputed data did not change these results.

### Table 2. Multivariable Linear Regression Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>25-Hydroxyvitamin D Effect, ng/mL (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily vitamin D supplementation (yes vs no)</td>
<td>3.4 (2 to 4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cow’s milk intake (per cup/d)</td>
<td>1.6 (1 to 2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Season (May-Sep vs Oct-Apr)</td>
<td>1.6 (0.4 to 2.8)</td>
<td>.004</td>
</tr>
<tr>
<td>Outdoor free play (per h/d)</td>
<td>0.3 (0 to 0.4)</td>
<td>.03</td>
</tr>
<tr>
<td>Skin pigmentation (Fitzpatrick type I-III vs IV-VI)</td>
<td>2.7 (1.2 to 4.4)</td>
<td>.001</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>-0.2 (-0.4 to 0)</td>
<td>.05</td>
</tr>
<tr>
<td>BMI z score (per unit)</td>
<td>-0.6 (-1.2 to 0)</td>
<td>.03</td>
</tr>
<tr>
<td>Screen time (per 1 h/d)</td>
<td>0.2 (0 to 0.4)</td>
<td>.22</td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index.

SI factor conversion: To convert 25-hydroxyvitamin D to nanomoles per liter, multiply by 2.496.

**COMMENT**

In this prospectively designed study, we have examined the contribution of 2 modifiable dietary determinants on 25-hydroxyvitamin D level in the context of known, and largely nonmodifiable, factors including...

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**Figure 2.** Distribution of study participants’ 25-hydroxyvitamin D serum levels. To convert 25-hydroxyvitamin D to nanomoles per liter, multiply by 2.496.
skin pigmentation and season in a population of healthy urban preschoolers at latitude 43.4°N over multiple seasons.

We have identified that vitamin D supplementation was associated with an increase in 25-hydroxyvitamin D level of approximately 3 ng/mL and vitamin D–fortified cow’s milk was associated with an increase in 25-hydroxyvitamin D level of approximately 2 ng/mL per 250-mL cup. In addition, these 2 modifiable variables appear to account for most of the explained variation in 25-hydroxyvitamin D level. The effects of vitamin D supplementation and cow’s milk intake on 25-hydroxyvitamin D level appear similar regardless of children’s skin pigmentation and season. We suggest that these findings are clinically meaningful and underscore the importance of dietary vitamin D intake to 25-hydroxyvitamin D status in early childhood.

Early childhood is a critical stage in human development with respect to the cumulative effects of both biologic and lifestyle determinants of health. Achieving and maintaining optimal vitamin D levels in early childhood may be relevant to health outcomes in later childhood and adulthood.\textsuperscript{15,37} Dietary records from Canadian infants have demonstrated that at 12 months of age, average daily intake of vitamin D from complementary food accounts for only 11% of the currently recommended dietary allowance of vitamin D.\textsuperscript{38,39} Therefore, other strategies to maintain 25-hydroxyvitamin D level as children transition from vitamin D–fortified formula or breast milk with vitamin D supplementation are necessary. To date, there has been limited understanding of modifiable influences on 25-hydroxyvitamin D level in early childhood. Our findings have practical implications for both clinical practice and health policy.

Our finding that variables reflecting cutaneous production of 25-hydroxyvitamin D (skin pigmentation, season, and outdoor play time) appear to explain less variation in 25-hydroxyvitamin D level than dietary intake variables in early childhood is consistent with

Figure 3. The effect of milk intake, skin pigmentation, vitamin D supplementation, and month on 25-hydroxyvitamin D serum levels. “Light skin pigmentation” represents Fitzpatrick skin pigmentation types I to III and “dark skin pigmentation” represents Fitzpatrick skin pigmentation types IV to VI. Effects adjusted for the average child (age, 37 months; body mass index z score = 0.2; 1 hour of outdoor play; and 1 hour of daily screen time). To convert 25-hydroxyvitamin D to nanomoles per liter, multiply by 2.496.
the findings of Gordon et al\textsuperscript{19} but may be in contrast to studies of older children, adolescents, and adults.\textsuperscript{40-42} We speculate that this could be a consequence of current cultural practice of sun avoidance of young children. If skin exposure to the sun is minimal, cutaneous production of 25-hydroxyvitamin D would also be expected to be minimal regardless of skin pigmentation, outdoor play time, or season.

Major strengths of this study include the relatively large sample size, which allowed sufficient statistical power to assess the independent effect of vitamin D supplementation and cow’s milk intake, availability of detailed clinical data, and a population of children with a high frequency of vitamin D supplementation. Previous studies examining predictors of vitamin D level in early childhood have been unable to examine the role of vitamin D supplementation because of the low frequency of supplement use.\textsuperscript{16,17,40,43} Limitations of this study include a single geographic location, although 43.4°N is at similar latitude to several other large North American cities. Additionally, the absence of geographic variability allowed us to study variables related to cutaneous vitamin D production independent of geographic variability. Another limitation is the effect of unmeasured confounders that may have introduced bias. These include the amount of vitamin D consumed in supplements, although all supplemental vitamin D in Canada marketed to children contains 400 IU/dose, as well as background vitamin D dietary intake aside from that provided through vitamin D–fortified cow’s milk and vitamin D supplementation. However, the absence of vitamin D fortification of foods other than milk in Canada and the generally low consumption of foods naturally rich in vitamin D by preschoolers (ie, fatty fishes) make this contribution likely to be small.\textsuperscript{21,38,44} Finally, as this was a cross-sectional study, causation cannot be inferred from the identified associations.

We have identified that 2 modifiable dietary intake variables (vitamin D supplementation and cow’s milk intake) are the most important determinants of 25-hydroxyvitamin D status in early childhood. Although excessive cow’s milk intake has been associated with iron deficiency,\textsuperscript{44} vitamin D supplementation and sensible cow’s milk intake represent excellent targets for increasing 25-hydroxyvitamin D level in young children.

Accepted for Publication: August 8, 2012. 
Published Online: January 14, 2013. doi:10.1001/2013.jamapediatrics.226

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Conflict of Interest Disclosures: None reported.

Funding/Support: Overall support for the TARGet Kids! program was provided by the Canadian Institutes of Health Research Institute of Human Development, Child and Youth Health and the Institute of Nutrition, Metabolism, and Diabetes as well as the St Michael’s Hospital Foundation. The Paediatric Outcomes Research Team is supported by a grant from The Hospital for Sick Children Foundation.

Role of the Sponsors: Funding agencies had no role in the design, collection, analyses, or interpretation of results of this study.

Additional Contributions: We thank Azar Azad, PhD, Tonya D’Amour, MSc, Kanthi Kavikondala, MSc, Tarandeep Malhi, MSc, Magda Melo, MSc, Subitha Rajakumar, BSc, Juela Sejdo, BSc, and Laurie
Thompson, BSc, for administrative and technical support for the TARGetKids! program.

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