Demographic, dietary, and biochemical determinants of vitamin D status in inner-city children

Thomas O Carpenter, Francisca Herreros, Jane H Zhang, Bruce K Ellis, Christine Simpson, Esther Torrealba-Fox, Grace J Kim, Mary Savoye, Nancy A Held, and David EC Cole

ABSTRACT
Background: Reports of clinical rickets are particularly evident in minority infants and children, but only limited analyses of vitamin D are available in this demographic group.

Objective: We sought to characterize circulating 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)2D], and their determinants, including circulating parathyroid hormone (PTH), total alkaline phosphatase activity (ALP), calcium, and phosphorus, in minority infants and children.

Design: We obtained demographic information and blood samples for measurement of PTH, ALP, 25(OH)D, and 1,25(OH)2D in >750 6-mo- to 3-y-old children. Dietary intake data were obtained and analyzed.

Results: The mean (±SD) 25(OH)D concentration was 66 ± 22 nmol/L (26.3 ± 8.7 ng/dL). A total of 15% of children had 25(OH)D concentrations less than the recommended target threshold of 50 nmol/L. Combined elevations of PTH and ALP occurred in only 2.5% of children. Determinants of 25(OH)D included vitamin D intake, age (decreasing with age), skin type (greater concentrations in lighter-skinned children than in darker-skinned children), formula use (higher intake), season (greater concentrations in the summer and fall than in the winter and spring), and, inversely, PTH. The mean 1,25(OH)2D concentration was 158 ± 58 pmol/L (60.6 ± 22.5 pg/mL), which was consistent with a reference range of 41–274 pmol/L or 15.7–105.5 pg/mL. Determinants for 1,25(OH)2D were age (decreasing with age), sex (greater concentrations in girls than in boys), skin type (greater concentrations in lighter-skinned children than in darker-skinned children), and, inversely, serum calcium and phosphorus.

Conclusions: Although 15% of subjects were vitamin D insufficient, only 2.5% of subjects had elevations of both PTH and ALP. The greater 25(OH)D concentrations observed with formula use confirm that dietary vitamin D fortification is effective in this demographic group. Circulating 1,25(OH)2D is higher in infants than in older children and adults and, in contrast to 25(OH)D, is not directly correlated with nutrient intakes. Am J Clin Nutr 2012;95:137–46.

INTRODUCTION

Much attention has been given to the vitamin D status of the US population. The serum 25(OH)D concentration has been accepted as the primary determinant of vitamin D status, and various studies reported concentrations of this metabolite in children (1–7). These data have led to various interpretations of what threshold concentration represents deficiency and what concentrations are optimal for overall health status. Nevertheless, data regarding measurements of 25(OH)D and their relation to functional outcomes are particularly limited in infants and toddlers (8–12). Such information is critical because overt manifestations of vitamin D deficiency are most often evident in this age group. During rapid growth, young children may develop striking skeletal deformities of rickets or symptomatic hypocalcemia. Detailed and reliable information regarding biochemical status is generally more difficult to obtain in large numbers of young children than in adults because sampling issues are more challenging. Moreover, in groups of children at risk of vitamin D deficiency, children from urban minority settings appear to be the most vulnerable.

Indeed, our own experience with overt rickets in minority children (13) prompted us to examine a large cross-sectional sample of children from the local catchment area of greater New Haven, Connecticut. We examined potential demographic, dietary, and biochemical determinants of both circulating 25(OH)D and 1,25(OH)2D concentrations, as well as for PTH, in both bivariate and multivariate regression models and assessed the frequency of biochemical measures associated with the occurrence of rickets in this population.

1 From the Departments of Pediatrics (Endocrinology) (TOC, FH, BKE, ET-F, GJK, MS, and NAH), Internal Medicine (Endocrinology) (CS), and Orthopaedics and Rehabilitation (TOC), Yale University School of Medicine, New Haven, CT; the Veterans Administration Cooperative Studies Program Coordinating Center, VA Connecticut Healthcare System, West Haven, CT (JHZ); and the Departments of Laboratory Medicine and Pathobiology, Medicine, and Genetics, University of Toronto, Toronto, Canada (DECC).
2 Address correspondence to TO Carpenter, Department of Pediatrics, Yale University School of Medicine, PO Box 208064, New Haven, CT 06520-8064. E-mail: thomas.carpenter@yale.edu.
3 No funding agency had any role in the design, implementation, analysis, or interpretation of the research.
4 Supported by the Gerber Foundation and made possible by the Yale Center for Clinical Investigation (supported by Clinical and Translational Science Award UL1 RR024139 from the National Center for Research Resources of the NIH). Support for data analysis was provided in part by the Thasher Research Fund (award 02829-4).
5 Abbreviations used: ALP, total serum alkaline phosphatase activity; PTH, parathyroid hormone; RD, registered dietitian; 1,25(OH)2D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

First published online December 14, 2011; doi: 10.3945/ajcn.111.018721.


Downloaded from ajcn.nutrition.org by guest on September 9, 2017
SUBJECTS AND METHODS

We expected that this proposed large sample would allow us to estimate the prevalence of vitamin D deficiency in healthy infants and toddlers in an inner-city minority population associated with the occurrence of overt rickets, and in which risk factors such as Northern latitude and skin pigmentation are present. We sought to characterize circulating 25(OH)D and 1,25(OH)2D concentrations and to assess other biochemical measures associated with rickets in these children. To this end, we selected measures (ie, serum PTH and ALP concentrations) that are typically in the markedly elevated range in patients with active overt rickets (14); we used minimal thresholds of elevation for both parameters to define at-risk cases. Because a variety of demographic features have been reported to be associated with vitamin D deficiency in older populations, we administered an extensive questionnaire aimed at capturing relevant demographic factors potentially associated with vitamin D status. We instructed all caregivers of the children in dietary diary methods and collected information on the dietary intake of each infant for 3 separate 24-h periods to identify important nutritional determinants of vitamin D status. We successfully enrolled >750 children over a 40-mo period. In this article, we report the results of this prospective study, including a characterization of the demographic, biochemical, and dietary variables that characterize infants.

Subjects

More than 750 healthy children aged 6–36 mo were sampled during well-child (nonillness related) visits to 4 neighborhood health clinics in the Greater New Haven area (CT). Exclusion criteria included 1) a history of disorders that affect vitamin D or mineral metabolism, 2) the use of medications known to affect vitamin D metabolism, including systemic glucocorticoid medications, and pharmacologic vitamin D metabolites such as calcitriol, 3) use of vitamin D supplements in excess of 400 IU/d, or 4) gestational age <28 wk. The study was approved by the Yale University Human Investigation Committee, and written informed consent was obtained from the appropriate parent or guardian of each child. All specimens and data were collected between May 2005 and July 2008.

Data collection

Demographic information was obtained from the primary caretaker of the child at the time of the visit. A detailed questionnaire was designed for the study, which identified socioeconomic variables, breastfeeding and formula-feeding history, and sunlight exposure (Table 1). Assessments of skin type were made by using the Fitzpatrick skin-pigmentation scale (16). One of 4 grades of pigmentation was recorded on the basis of a comparison with a color chart simultaneously examined at the interview. Ancestry was ascertained by obtaining a self-report of ancestry and/or origin of all 4 grandparents. To be categorized in any of the groups, 3 of the 4 grandparents were reported to be of that group. If there were not 3 grandparents who reported to be of the same ethnicity, the subject was classified as “other” on the basis of the likely admixture.

Detailed dietary intakes were recorded by the caregivers after careful instructions were provided for the identification of food types and quantities. A 3-d food record was kept and a multiple pass interview (17) was used by research RDs and RD-trained research assistants to minimize omissions of food and beverages. Caregivers recorded 2 weekdays and 1 weekend day of intake.
and RDs quickly followed up with a scheduled telephone interview by using the multiple-pass technique of several related questions (eg, amounts, preparation method, and addition of condiments). RDs with experience in the Nutrition Data Software for Research program (NDS-R, version 2006; University of Minnesota) entered the 3-d food records and calculated the average daily intake of nutrients. Although there were very few breastfed infants (<2%), and they all consumed food as well, the technique of volume per minute (18) was used to capture the nutrient intake of breast milk because this was practical for the study and its population and provided a standardized method of quantification. Average daily intakes of nutrients of interest to skeletal health, including calcium, magnesium, and vitamin D (from food plus supplements), were estimated, as was mean daily protein and total caloric intakes. A blood sample was obtained for subsequent measurement of serum calcium, phosphorus, ALP, PTH, 25(OH)D, and 1,25(OH)2D.

Analytic methods

Serum minerals and ALP determinations were performed by the Clinical Chemistry Laboratory, Yale-New Haven Hospital. Total serum calcium was determined by using flame-atomic absorptiometry (model 2380; PerkinElmer). Serum phosphorus and ALP were measured by using a Roche Diagnostics DPP modular autoanalyzer (Roche Diagnostics). Serum 25(OH)D and 1,25(OH)2D were measured by using radioimmunoassay kit methodology (DiaSorin). Results of samples analyzed in our assay for serum 25(OH)D were consistently shown to agree with the midrange of outcomes of individuals who used this assay and participated in the international Vitamin D External Quality Assessment Scheme standardization system (19). The interassay and intraassay CVs for our 25(OH)D assay were 9.6% and 6.6%, respectively. Serum PTH was measured with antisera to the midregion of human PTH by using the 125I-labeled 37–84 residue fragment of bovine PTH as radioactive trace, as previously described (20). The interassay and intraassay CVs for our PTH assay were 13.1% and 15.0%, respectively.

Data analysis

Children with elevated values for both PTH and ALP together were classified as being at risk of development of rickets. ALP values >350 IU/L were considered elevated, which was a threshold established by our analysis of the distribution of ALP concentrations reported in 6023 1–3-y-old children at Yale-New Haven Hospital (see supplementary figure under “Supplemental data” in the online issue). Analysis of this distribution revealed rightward skewness, with a mean (±SD) of 247 ± 179 IU/L and median of 210 IU/L. We estimated from visual inspection that the right tail of this distribution would fall between 300–400 IU/L. Thus, on the basis of this observation (for which we used the assay also used in the current study), as well as accumulated reports, we considered an ALP concentration >350 IU/L as elevated.

For PTH, we used a threshold of 25 nLEq/mL, which is the historically established upper limit of normal for this assay (20) and has been observed in clinical use for children and young adults. The combination of increased values for these 2 biomarkers PTH, identified as the earliest consistent biochemical change in the evolution of rickets (14), and ALP, which represents a sufficient duration of a rachitogenic process to affect the skeleton, was used as the physiologic indicator of impaired mineral homeostasis that, if allowed to progress, would result in overt rickets. Children identified as at risk of rickets by this criterion were referred to their primary health care provider for additional evaluation. We recommended that children identified as at risk be supplemented daily with calcium (30 mg elemental calcium/kg) and, in addition, with vitamin D (400 IU) if the serum 25(OH)D concentration was <16 ng/mL and follow-up biomarkers be repeated 3 mo later. Follow-up calls to care providers of identified children were made to assess the outcome and obtain results of any follow-up laboratory values.

Descriptive statistics and box plots were used to summarize data. Pearson’s correlation was used to evaluate the association between biochemical laboratory and nutrition measurements. ANOVA and analysis of regression were performed to assess the relative contributions of age, ethnicity, skin type, and other demographic factors to circulating 25(OH)D, 1,25(OH)2D, and PTH concentrations. Multivariate regression analysis was performed to determine significant demographic, biochemical, and nutritional determinants of circulating 25(OH)D and 1,25(OH)2D concentrations. We used a 0.05 significance level on the basis of 2-tailed tests in all cases. SAS software (version 9.2; SAS Institute Inc) was used for all analyses.

RESULTS

Characteristics of subjects

Results were available for a total of 781 subjects. Demographic characteristics of the subjects, as reported by the responses of caretakers to the questionnaire or as ascertained by the interview, are shown in Table 1. Subjects ranged from 6 to 36 mo of age (mean ± SD: 21.2 ± 8.9 mo of age). Mean body weight at the time of the visit was 11.8 ± 2.8 kg. Of the 4 skin-type categories (light skinned, slightly pigmented, moderately dark skinned, and very dark-skinned), one-fourth of the children were light skinned, and the remainder of children were classified as one of the 3 darker categories of skin pigmentation. Ancestry (as previously defined) was largely Hispanic (64%) and African American (23%) with relatively few whites (2%).

Of 526 children that had been breastfed as a sole nutritional source, nearly one-third of children discontinued breastfeeding by 1 mo of age and over one-half of children discontinued breastfeeding by 2 mo of age. All children had ceased breastfeeding as the primary source of nutrition by 5 mo age. At the time of study, most children (84%) were no longer fed formula, and 95% of children were reported to have discontinued formula feeding by 1 y of age.

Biochemical profile

Distribution profiles of measured biochemical variables are shown in Figure 1. The mean (±SD) serum 25(OH)D concentration was 66 ± 22 nmol/L (26.3 ± 8.7 ng/dL), which was well within the sufficient range recommended by the recent Institute of Medicine report on Dietary Reference Intakes for calcium and vitamin D (15). The geometric mean serum 25(OH)D concentration was 63 nmol/L (25 ng/dL), which was in keeping with
the somewhat skewed distribution. A reference interval (calculated as the mean $\pm 2$ SDs) was 22–110 nmol/L (9–44 ng/dL). Approximately 1 in 7 children ($n = 113; 15\%$) had circulating $25$(OH)$_2$D concentrations $<50$ nmol/L (20 ng/mL) and $\sim 1$ in 17 children ($n = 44; 5.8\%$) had circulating $25$(OH)$_2$D concentrations $<40$ nmol/L (16 ng/mL).

The mean serum calcium concentration was $10.0 \pm 0.5$ mg/dL (reference interval: 9.1–11.0 mg/dL). The mean serum phosphorus concentration was $5.4 \pm 0.6$ mg/dL (reference interval: 4.2–6.6 mg/dL). The mean ALP concentration was $306 \pm 401$ IU/L, its geometric mean was 260 IU/L, and the calculated reference interval was 100–614 IU/L. The frequency distribution of serum PTH was also skewed rightward, as were serum 1,25(OH)$_2$D values. The geometric mean of PTH values was 17.8 nLEq/mL (interval: 15.5–55.7 nLEq/mL). On the basis of our independent reference data for this assay, 22% of individuals had elevations in their PTH concentration. The mean serum 1,25(OH)$_2$D concentration was $158 \pm 58$ pmol/L (61 ± 22 pg/mL), and the reference interval was 41–274 pmol/L (16–106 pg/mL).

Establishment of cases at risk of developing rickets

Surprisingly, only 19 children (2.5%) met our a priori criteria for being at risk of developing rickets, despite a greater prevalence of vitamin D insufficiency and deficiency in the entire study population (see Biochemical profile). In all 19 cases in which the combination of elevated ALP and PTH were identified, we contacted the referring health provider and recommended calcium supplementation, and if the $25$(OH)$_2$D concentration was $\leq 37.5$ nmol/L (15 ng/mL), we recommended vitamin D supplementation. Repeat PTH measures were performed in 8 cases after supplementation, and repeat ALP measurements were performed in 9 cases after supplementation. The previously elevated PTH and ALP values decreased in all cases measured (AP normalized entirely in 6 cases, and PTH normalized entirely in 4 cases), thereby validating these measures as a reasonable clinical index.

We wished to identify whether this risk of development of rickets correlated with vitamin D deficiency, and therefore, we examined the frequency of elevations in PTH and alkaline phosphatase with respect to various thresholds of $25$(OH)$_2$D concentrations. Our a priori definition of risk of developing rickets used the combination of PTH and ALP elevations. With a review of the correlation analysis between serum ALP and $25$(OH)$_2$D (Pearson’s correlation: $-0.03; P = 0.37$) and between serum PTH and $25$(OH)$_2$D (Pearson’s correlation: $-0.06; P = 0.09$), we noted the absence of significant associations of $25$(OH)$_2$D with PTH or ALP. However, for the prediction of
thresholds of 25(OH)D associated with risk of rickets, we examined elevations in PTH concentrations because, we found a significant association of 25(OH)D and PTH. For various 25 (OH)D thresholds, we observed a consistently higher rate of vitamin D deficiency in patients with elevated PTH than in patients without elevated PTH. Of the various thresholds examined (ie, 25, 40, 50, and 75 nmol/L), we chose 40 nmol/L as identifying the threshold concentration of 25(OH)D associated with an elevation in PTH because of the relatively better sensitivity (0.1) and specificity (0.94) at this threshold than that at all of the other thresholds.

Nutritional profile

Daily dietary calcium, magnesium, and protein (adjusted for body weight) and total vitamin D intake from food and supplements are shown in Figure 2. The mean daily caloric intake, which was expressed per body weight, was 1038 ± 328.4 kcal/kg, and the mean overall vitamin D intake from food plus supplements was 249 ± 129 IU/d (as shown with means of other nutrients in Table 2). The proportion of children who met the current recommendations for Dietary Reference Intakes (15) are shown in Table 2.

Analysis of potential determinants of vitamin D or PTH status

Demographic factors

Circulating 25(OH)D decreased with age (Figure 3). Regression analysis indicated that this decrease was significant; the magnitude of the decrement was 0.44 nmol · L⁻¹ · mo⁻¹ or 5.3 nmol · L⁻¹ · y⁻¹. Race and season of sampling were expected determinants of the circulating 25(OH)D concentration (Figure 4). Individuals identified as white had the greatest 25(OH)D values, followed by Hispanics and African Americans, respectively (P = 0.0055). Specifically, concentrations in white subjects were greater than in subjects of African ancestry (P = 0.049), and values in Hispanic subjects were greater than in those in subjects of African ancestry (P = 0.002). Skin type was also significantly related to 25(OH)D (P = 0.008); however, skin type and race were closely related to one another (P < 0.001). Subjects sampled in the winter or spring had lower 25(OH)D concentrations than did subjects sampled in the summer or fall (P = 0.001), although the magnitude of this seasonal difference was small (~6.8 nmol/L or 2.7 ng/mL), and there was no relation to seasonal sun exposure (Table 3, footnote 1). All demographic factors associated with circulating 25(OH)D concentrations are shown in Table 3. In addition to age, race, and season of sampling, significant correlates included skin type, home ownership, and the number of people or children in the household. Several parameters related to past or current use of formula were also significantly associated with 25(OH)D concentrations. In particular, subjects who were currently receiving formula, subjects who previously used formula, and the use of formula through a later age (ie, cessation of formula use at an older age of the child) were all associated with higher circulating 25(OH)D concentrations. Because 25(OH)D concentrations decreased with age, and fewer children received formula at older ages, we considered that age confounded the effect of formula. However, when the model was adjusted for age, the effect of formula feeding at the time of the sampling remained significant (P < 0.030). We did not show a consistent relation of circulating 25(OH)D and seasonal sunlight exposure.

As with 25(OH)D, circulating 1,25(OH)₂D decreased with age (Figure 3). Regression analysis indicated that this decrease was significant and with a much larger relative change than that for 25(OH)D. The monthly decrement was 1.5 pmol 1,25(OH)₂D/L or 18 pmol 1,25(OH)₂D/L [7 pg 1,25(OH)₂D/mL] per year. Other demographic factors shown to be associated with circulating 1,25(OH)₂D included race and sex (Figure 4). African Americans had significantly higher 1,25(OH)₂D values than did Hispanics (P = 0.001), who had comparable concentrations to whites. Girls had higher 1,25(OH)₂D concentrations than did boys (P = 0.038). As with 25(OH)D, the current use of formula was associated with higher 1,25(OH)₂D concentrations (Table 3). Skin type was also significantly related to 1,25(OH)₂D (P <
Correlation analysis of serum 25(OH)D, serum 1,25(OH)2D, and PTH with all other biochemical variables is shown in Table 4. Serum 25(OH)D concentrations were positively correlated with serum calcium and serum phosphorus (Pearson’s correlation: 0.154; P = 0.002), and moderately correlated with the total vitamin D intake from food and supplements (Pearson’s correlation: 0.154; P < 0.001) and with weight-adjusted daily intakes of dietary calcium (P = 0.002), phosphorus (P = 0.002), and magnesium (P = 0.013). Serum 1,25(OH)2D had no relation to any of these dietary variables.

Multivariate analysis

A multivariate analysis was performed to identify determinants of each of the 2 vitamin D measures (serum 25(OH)D and 1,25(OH)2D) as well as for serum PTH. With consideration of each measure as a dependent variable, we modeled separate

TABLE 2
Summary of daily dietary intake

<table>
<thead>
<tr>
<th>Dietary variable</th>
<th>All subjects</th>
<th>6–12 mo old (n = 131)</th>
<th>1–3 y old (n = 645)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal/kg BW)</td>
<td>1038 ± 328.4</td>
<td>836 ± 242.8***</td>
<td>1107 ± 328.6</td>
</tr>
<tr>
<td>Protein (g/kg BW)</td>
<td>3.3 ± 1.3</td>
<td>2.8 ± 1.4***</td>
<td>3.4 ± 1.2</td>
</tr>
<tr>
<td>Calcium (mg/kg BW)</td>
<td>66 ± 32</td>
<td>74 ± 32.4 (94%, 260 mg/d)**</td>
<td>64 ± 31.4 (54%, 700 mg/d)</td>
</tr>
<tr>
<td>Phosphorus (mg/kg BW)</td>
<td>67 ± 25.9</td>
<td>62 ± 29.7 (87%; 275 mg/d)**</td>
<td>68 ± 25.0 (94%, 460 mg/d)</td>
</tr>
<tr>
<td>Magnesium (mg/kg BW)</td>
<td>12 ± 4.3</td>
<td>12 ± 5.1 (76%, 75 mg/d)</td>
<td>12 ± 4.2 (95%, 80 mg/d)</td>
</tr>
<tr>
<td>Vitamin D (IU/d)</td>
<td>249 ± 128.6</td>
<td>290 ± 124.0 (82%, 400 IU/d)***</td>
<td>240 ± 128.0 (2%, 600 IU/d)***</td>
</tr>
</tbody>
</table>

* Younger group (column 2) compared with older group (column 3): ***P < 0.001, **P = 0.01, *P < 0.05. 

1 * Mean ± SD (all such values).

2 The percentage of children who met the age-specific Dietary Reference Intake recommendations (15) and the Adequate Intake are shown in parentheses.

3 The percentage of children who met the age-specific Dietary Reference Intake recommendations (15) and the Recommended Daily Allowance are shown in parentheses.

A multivariate analysis was performed to identify determinants of each of the 2 vitamin D measures (serum 25(OH)D and 1,25(OH)2D) as well as for serum PTH. With consideration of each measure as a dependent variable, we modeled separate

TABLE 3
Demographic factors that correlated with vitamin D or PTH status

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Estimator</th>
<th>Direction</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>−0.44</td>
<td>Decreases with age</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race</td>
<td>8.3, 5.3 (AA is reference)</td>
<td>W &gt; H &gt; AA</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Skin Type</td>
<td>−1.97</td>
<td>Lightest &gt; darkest</td>
<td>0.008</td>
</tr>
<tr>
<td>Season of sampling</td>
<td>4.7</td>
<td>Summer and fall &gt; winter and spring</td>
<td>0.001</td>
</tr>
<tr>
<td>Home ownership</td>
<td>−6.18</td>
<td>Own &gt; rent</td>
<td>0.036</td>
</tr>
<tr>
<td>Number of people in household</td>
<td>−0.819</td>
<td>Fewer &gt; more2</td>
<td>0.024</td>
</tr>
<tr>
<td>Number of children in family</td>
<td>−0.023</td>
<td>Fewer &gt; more2</td>
<td>0.011</td>
</tr>
<tr>
<td>Use of formula in first 6 mo of life</td>
<td>4.9</td>
<td>Yes &gt; no</td>
<td>0.029</td>
</tr>
<tr>
<td>Currently formula feeding</td>
<td>8.15</td>
<td>Yes &gt; no</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at cessation of formula</td>
<td>3.16</td>
<td>Decreases with age</td>
<td>0.021</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>−1.5</td>
<td>Decreases with age</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>9.58</td>
<td>Girls &gt; boys</td>
<td>0.038</td>
</tr>
<tr>
<td>Currently formula feeding</td>
<td>16.59</td>
<td>Yes &gt; no</td>
<td>0.008</td>
</tr>
<tr>
<td>Race</td>
<td>−10.9, −24.5 (AA is reference)</td>
<td>AA &gt; W &gt; H</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Skin type</td>
<td>9.9</td>
<td>Darkest &gt; lightest</td>
<td>0.008</td>
</tr>
<tr>
<td>PTH</td>
<td>−6.03, −1.22 (AA is reference)</td>
<td>AA &gt; H &gt; W</td>
<td>0.018</td>
</tr>
</tbody>
</table>

1 Demographic factors that were not significant correlates of PTH or vitamin D metabolite concentrations included type of home, birth order, primary wage earner, mother’s current smoking status, age at which nursing started or stopped, current pregnancy status of the mother, sun exposure, extent of skin covered by maternal dress, age at first tooth eruption, or age when formula was introduced. AA, African American; H, Hispanic; PTH, parathyroid hormone; W, white; 1,25(OH)2D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

2 Households and families with fewer numbers of people and children had higher concentrations of 25(OH)D; 1,25 (OH)2D, 1,25-dihydroxyvitamin D.
analyses on the basis of the nature of the potential determinants. That is, demographic variables comprised one analysis, biochemical variables comprised a second analysis, and nutritional variables comprised another analysis (see models 1–3 in supplementary Table 1 under “Supplemental data” in the online issue) so as not to mix disparate categories of data. After these analyses, we included all demographic, biochemical, and nutritional variables into a combined model for each of the dependent variables. The significant determinants of the respective vitamin D metabolites are shown in Table 5. For 25(OH)D, significant determinants included vitamin D intake, age (younger children had greater concentrations), season, formula use in the first 6 mo of life (which resulted in higher 25(OH)D concentrations), skin type (lighter-skinned children had greater concentrations), and serum PTH [higher PTH concentrations were associated with lower 25(OH)D concentrations]. For 1,25(OH)2D, skin type (darker-skinned children had greater concentrations), serum phosphorus [lower phosphorus concentrations were associated with greater 1,25(OH)2D concentrations], age (younger children had greater concentrations), sex (girls had greater concentrations than did boys), and serum calcium (lower calcium concentrations were associated with greater 1,25(OH)2D concentrations) remained significant determinants in the combined multivariate model. For PTH, 25(OH)D was inversely associated with PTH, and only caloric intake was positively associated with PTH.

DISCUSSION

Nutritional rickets and vitamin D status in at-risk children are of continuing concern. We investigated a large cohort with a wide profile of demographic factors (Table 1). The mean age of subjects was 21 mo, which was comparable with the mean age of children with overt rickets from this population of 20 mo (13). We used strict criteria for the categorization of ancestry (by using the reported ethnicity of 3 of the 4 biological grandparents) to limit potential effects of admixture on outcomes. The 10% of subjects identified as other primarily represented a mixed-ancestry group (the absence of 3 similarly identified grandparents). We rigorously ascertained dietary nutrient intakes after providing quantitative, illustrated instructions to caregivers. Circulating 25(OH)D was determined by using a monitored, standardized assay, and subclinical rickets was assessed by using validated biomarkers. This cohort provided generalizable data for inner-city children in Northern US latitudes, which is the demographic group most at risk of rachitic bone disease. Finally, we assessed circulating mineral and 1,25(OH)2D concentrations because available data for this age group are relatively sparse.

Vitamin D status

Approximately 6% of children manifest serum 25(OH)D concentrations <40 nmol/L (16 ng/mL), which is the threshold suggested by our analysis as the most appropriate for identification of elevated PTH concentrations. Fifteen percent of children had circulating 25(OH)D concentrations <50 nmol/L (20 ng/mL), which is the target concentration recommended in the recent Institute of Medicine report (15). Overall, our data support the notion outlined in that report that overt skeletal changes are generally not evident in children when circulating 25(OH)D concentrations are >40–50 nmol/L. Thus, the suggested target
concentration for 25(OH)D of ≥50 nmol/L appears consistent with the maintenance of skeletal health, at least in young children.

Determinants of vitamin D

25(OH)D

Our regression and correlative analyses of circulating 25(OH)D and 1,25(OH)₂D concentrations identified interesting potential determinants of these measures. Although circulating 25(OH)D has been recently reported to decrease with age in older children (21), to our knowledge, this finding in infants is novel. Expected differences related to race and skin pigmentation were evident, and whites and the least-pigmented individuals had the highest concentrations. The seasonal difference in 25(OH)D was more modest than anticipated, and we could not show a correlation of time spent outdoors, times of day spent outdoors, or covering of exposed skin with 25(OH)D in children sampled in either the winter and spring or summer and fall seasons. Socioeconomic factors such as the number of people (or children) in the household and home ownership correlated with 25(OH)D concentrations. Indeed, a household-visit study of rickets in Bangladesh identified household size and other socioeconomic factors as significant correlates of the disease (22). Nutrition-related correlates of 25(OH)D included the use of infant formula in the first 6 mo of life, current use of formula, or a later age when formula feeding was discontinued. Although serum 25(OH)D correlated with daily intakes of calcium, phosphorus, and magnesium, it was most strongly correlated with the total daily intake of vitamin D. Experimental animal models with low calcium intakes showed increased clearance of 25(OH)D (23), which is perhaps the mechanism responsible for the association of 25(OH)D concentrations and dietary calcium intake. Because
That was opposite that observed for 25(OH)D. Concentrations of 1,25(OH)_{2}D showed a modest negative correlation with serum phosphorus, which was consistent with the well-known effects of serum phosphorus on the regulation of the vitamin D 1α-hydroxylase. Finally, the strong positive correlation between circulating 25(OH)D and 1,25(OH)_{2}D may have reflected the loose regulation of 1α-hydroxylase in young children and the dependence of the 1,25(OH)_{2}D concentration on the availability of its substrate 25(OH)D. Unlike 25(OH)D, serum 1,25(OH)_{2}D did not correlate with dietary intake variables, which was consistent with the general consideration that serum concentrations of this metabolite are related more to homeostatic control than to dietary influences. With multivariate analysis, we showed that 1,25(OH)_{2}D was significantly determined by age, sex, skin type, and serum calcium (Table 5).

This study provided a comprehensive analysis of the determinants of circulating 25(OH)D and 1,25(OH)_{2}D concentrations in a population at risk of the development of vitamin D–deficiency rickets. Reference data from the study should be particularly applicable in the clinical setting as applied to groups with demographic features similar to our cohort. To our knowledge, novel determinants of circulating 25(OH)D concentrations identified in this study included age and exposure to infant formula. We confirmed anticipated effects of race, skin type, and socioeconomic variables on 25(OH)D. Furthermore, we identified correlates of circulating 1,25(OH)_{2}D, and showed its expected association with biochemical rather than nutritional variables. The importance of total vitamin D intake, which was reflected in part by the positive effect on 25(OH)D of infant formula (fortified with vitamin D), provides evidence at the population level that supplementary vitamin D practices are likely to be effective in increasing 25(OH)D concentrations in young children. We encountered very few subjects (2.5%) with combined elevations in serum PTH and ALP, and yet, 15% of children had concentrations below the threshold of an adequate 25(OH)D concentration suggested by the Institute of Medicine committee report on vitamin D. Our data support the current target thresholds suggested by this committee for vitamin D sufficiency as related to bone health for this age group (15) and should be useful in the identification of strategies for the optimization of vitamin D nutrition in populations at risk of nutritional rickets.

We are grateful for the support of the Gerber Foundation and the Thrasher Research Fund and also acknowledge the support and cooperation of the following individuals who made this project possible: Elizabeth Bailey and Mary Ellen Flaherty-Hewitt at the Hospital of St Raphael, Robert Windham and Steven Updegrove at the Hill Health Center, Brian Forsyth of the Yale-New Haven Hospital Primary Care Clinic, Semeon Tsalbins of the Fair Haven Health Center. We thank Iris Maldonado for her dedicated assistance, and the families of the children for their generosity in participating in the study.

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>Alkaline phosphatase</th>
<th>PTH</th>
<th>25(OH)D</th>
<th>1,25(OH)_{2}D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.248**</td>
<td>0.009</td>
<td>0.141**</td>
<td>-0.047</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>-0.035</td>
<td>-0.041</td>
<td>0.158**</td>
<td>-0.096*</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>-0.030316</td>
<td>-0.033</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>-0.060*</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D</td>
<td></td>
<td>0.153**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Willis CM, Laing EM, Hall DB, Hausman DB, Lewis RD. A prospective analysis of plasma 25-hydroxyvitamin D concentrations in...


