Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents[1–3]

Francis L Weng, Justine Shults, Mary B Leonard, Virginia A Stallings, and Babette S Zemel

ABSTRACT

Background: Serum 25-hydroxyvitamin D [25(OH)D] concentrations serve as a biomarker for vitamin D stores. Prior studies have not examined the risk factors for low vitamin D concentrations in a multiethnic sample of US youth across a broad age range.

Objective: The objective was to determine the prevalence of and factors associated with low concentrations of 25(OH)D in children and adolescents.

Design: Serum 25(OH)D concentrations were measured in 382 healthy children aged 6-21 y living in the northeastern United States. Dietary and supplemental vitamin D intake was assessed by interview. Fat and lean mass were assessed by dual-energy X-ray absorptiometry. Multivariable ordinal logistic regression was used to determine factors associated with decreased concentrations of 25(OH)D.

Results: The median concentration of 25(OH)D was 28 ng/mL (interquartile range: 19–35 ng/mL), and 55% of subjects had 25(OH)D concentrations <30 ng/mL. 25(OH)D concentrations were inversely correlated with parathyroid hormone concentrations (Spearman's $r = -0.31$, $P < 0.001$) but were not significantly correlated with 1,25-dihydroxyvitamin D concentrations. In the multivariable model, older age ($P < 0.001$), black race [odds ratio (OR): 14.2; 95% CI: 8.53, 23.5], wintertime study visit (OR: 3.55; 95% CI: 2.29, 5.50), and total daily vitamin D intake <200 IU (OR: 1.58; 95% CI: 1.02, 2.46) were associated with low vitamin D concentrations. Fat and lean mass were not independently associated with vitamin D status in this healthy-weight sample.

Conclusion: Low serum 25(OH)D concentrations are prevalent in otherwise healthy children and adolescents in the northeastern United States and are related to low vitamin D intake, race, and season. *Am J Clin Nutr* 2007;86:150–8.

KEY WORDS Vitamin D, 25-hydroxyvitamin D, children, adolescents, 1;25-dihydroxyvitamin D, parathyroid hormone

INTRODUCTION

It is well recognized that adequate stores of vitamin D are crucial for musculoskeletal health (1, 2). The best indicator of vitamin D stores is the serum concentration of calcidiol, or 25-hydroxyvitamin D [25(OH)D] (3, 4). When circulating 25(OH)D concentrations are inadequate, a state known as hypovitaminosis D, intestinal calcium absorption and bone mineralization are impaired. More severe deficits in 25(OH)D lead to clinical myopathy, osteomalacia in adults, and rickets in children (5). A recent randomized clinical trial of vitamin D supplementation in healthy school-aged girls in Lebanon showed significant beneficial effects on lean mass and bone mineral content, especially during the premenarcheal period (6). In Finnish military recruits, stress fractures were associated with low vitamin D status (7). In addition to its musculoskeletal effects, vitamin D is important for immune function, and hypovitaminosis D may contribute to varied diseases, such as hypertension, cancer, multiple sclerosis, and type 1 diabetes (2).

Hypovitaminosis D remains an underrecognized problem in the general population and is poorly defined in children. Recent studies showed inadequate circulating 25(OH)D concentrations in adult medical inpatients (8), postmenopausal women (9), and free-living adults (10). In the pediatric population, several studies documented low serum vitamin D concentrations in adolescents living in Boston, Cleveland, and Maine (11–13), in infants and toddlers (14) in Alaska, and in children of primary school age in Lebanon (15). Of substantial concern, given the current obesity epidemic, is that obesity in children was also shown to be associated with decreased 25(OH)D concentrations (11, 16); however, these prior studies determined obesity by using body mass index (BMI; in kg/m^2) rather than a more direct estimate of body fat mass. In addition, prior studies did not examine the relations between vitamin D status, race, body composition, and dietary intake in children across a broad age range. The aims of this study were to determine *1*) the prevalence of serum 25(OH)D concentrations <30 ng/mL—a recognized indicator of hypovitaminosis D in adults (17) and of more severe deficits of 25(OH)D in children and adolescents—and *2*) the factors associated with reduced 25(OH)D concentrations.

[1] From the Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA (FLW); the Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA (FLW, JS, and MBL); and the Divisions of Nephrology (MBL) and Gastroenterology, Hepatology and Nutrition, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA (BSZ and VAS).

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[3] Address reprint requests to BS Zemel, Nutrition and Growth Laboratory, Division of Gastroenterology, Hepatology and Nutrition, The Children's Hospital of Philadelphia, 3535 Market Street, Room 1560, Philadelphia, PA 19104-4399. E-mail: zemel@email.chop.edu.

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SUBJECTS AND METHODS

Study design and participants

A cross-sectional study of skeletal development in healthy children aged 6-21 y from the Philadelphia, PA, area (at latitude 40°N) was conducted, and blood samples were obtained in a subset of the participants. Study participants were recruited through newspaper advertisements, mailings, and fliers to primary care centers and pediatric practices affiliated with The Children's Hospital of Philadelphia and the surrounding community. For inclusion, children had to have a reported height, weight, and BMI (at the time the appointment was scheduled) within the 5th to 95th percentiles (18). Children were excluded if they used any medications or had any chronic medical conditions that might affect growth, body composition, dietary intake, or physical activity.

The protocol was approved by the Committee for the Protection of Human Subjects. For study participants <18 y of age, written informed consent was obtained from a parent or guardian and verbal assent from the subject; written informed consent was obtained from participants ≥18 y of age.

Vitamin D metabolites and related measures

A nonfasting blood sample was drawn between 0800 and 1700 to determine serum concentrations of 25(OH)D, $1,25(OH)_2D$, parathyroid hormone (PTH), and bone-specific alkaline phosphatase (BSAP). The serum was stored in aliquots at −70 °C and shipped in batches for analysis (Quest Diagnostics' Nichols Institute, San Juan Capistrano, CA). Serum 25(OH)D was analyzed by ^{125}I-labeled radioimmunoassay with a commercially available test kit (DiaSorin, Stillwater, MN) (19). The DiaSorin primary antibody showed equal reactivity with $25(OH)D_2$ and $25(OH)D_3$ and excellent correlation with HPLC, the gold standard method (20). The intra- and interassay CVs were 2.2% and 8.6%, respectively. The $1,25(OH)_2D$ concentrations were determined by a radioreceptor assay that uses solid-phase extraction followed by competitive binding with calf thymus vitamin D receptor (21); the intra- and interassay CVs were 7-11% and 12-15%, respectively. Intact PTH concentrations were measured with the Nichols chemiluminescence assay (interassay CV: 7–9%), and serum BSAP was measured with a specific 2-site immunoradiometric assay (interassay CV: 8.5%).

For purposes of the analysis, we defined hypovitaminosis D as 25(OH)D concentrations <30 ng/mL, because this is a recognized cutoff for healthy vitamin D concentrations (5, 17). Study subjects were further categorized into 4 vitamin D status groups on the basis of 25(OH)D concentrations (<10, 10 to<20, 20 to <30, and ≥30 ng/mL). These cutoffs were used in a previously published study (22). In addition, the cutoffs of 20 and 30 ng/mL ensured that there were adequate numbers of study subjects in each category of vitamin D status (<20, 20 to <30, and ≥30 ng/mL) for the multivariable ordinal logistic regression analysis.

The reference ranges reported by the Nichols laboratory are as follows: for intact PTH the normal ranges are 9–59 pg/mL for ages 6-9 y, 11–74 pg/mL for ages 10-13 y, 9–69 pg/mL for ages 14-17 y, and 10–65 pg/mL for ages >17 y. For $1,25(OH)_2D$ the reference ranges are 27–71 pg/mL for ages 3-17 y and 16–60 pg/mL for ages >17 y. For BSAP, the reference ranges for female children and adolescents are 41.0-134.6 μg/L for ages 6-9 y, 24.2-154.2 μg/L for ages 10-13 y, 10.5-75.2 μg/L for ages 14-17 y, and 3.9-15.1 μg/L for ages >17 y. For male children and adolescents, the BSAP reference ranges are 41.0-134.6 μg/L for ages 6-9 y, 43.8-177.4 μg/L for ages 10-13 y, 13.7-128.0 μg/L for ages 14-17 y, and 5.9-22.9 μg/L for ages >17 y.

Growth, puberty status, and body composition

The height and weight of the subjects (in lightweight clothing and with shoes and adornments removed) were assessed with standard techniques (23). Age- and sex-specific SD scores (*z* scores) for BMI, height, and weight were calculated by using national reference standards (18). Sexual maturation was determined with a self-assessment pictorial questionnaire (24) that illustrated the 5 stages of development as described by Tanner (25). The self-assessment examination was carried out in a private room equipped with a mirror for self-examination, and assistance from the parent or guardian was available if needed. This self-assessment questionnaire was validated in our laboratory for children with Crohn disease (26).

Dietary intake

Dietary intakes of calcium and vitamin D were assessed via three 24-h recall interviews conducted by a research dietitian within 3 wk of the study visit. The first interview was conducted in person during the study visit, and the other 2 interviews were conducted by telephone. Depending on the age of the child, the parent or guardian and the child were interviewed with the use of food models, portion booklets, or serving containers to assist in estimating serving size. Nutrient analysis was performed with the Minnesota Nutrition Data System (University of Minnesota, Minneapolis, MN). For the subjects reporting the use of dietary supplements, the calcium and vitamin D contents from supplement sources were recorded during the study interview and, when necessary, confirmed by telephone after the study visit. Dietary and supplemental vitamin D and calcium intakes were compared with the Adequate Intakes as described in the Dietary Reference Intakes for these micronutrients (27) to calculate a percentage of the Adequate Intake [(dietary intake/Adequate Intake) × 100]. Of note, the Adequate Intake of vitamin D is 200 IU/d and of calcium is 800 mg/d in children aged 4-8 y, 1300 mg/d in children aged 9-18 y, and 1000 mg/d in adolescents and young adults aged >18 y.

Body composition

Fat mass and lean body mass were determined by dual-energy X-ray absorptiometry (DXA). Whole-body DXA scans (software analysis version 12.3; Hologic Discovery, Bedford, MA) were obtained in the array mode following standard positioning techniques. Children wore hospital scrubs to eliminate clothing artifacts. In our laboratory, the long-term in vitro CV for bone mineral density is <0.6%, and the in vivo CV is <1% (28). One investigator (BSZ) reviewed all scans to determine acceptability. The total body fat mass, excluding the head, was used in the analyses.

Fat mass is known to show age- and sex-related trends and to differ by ethnicity (blacks compared with nonblacks) (29). As has been suggested for adults and children, height is the appropriate measure for determining relative fatness (30, 31). In the absence of national reference data for total body fat measures, we used the method of Altman (32) to generate age-, sex-, and ethnicity-specific *z* scores for fat mass relative to height and for

lean body mass relative to height on the basis of the distribution within the sample.

Demographic characteristics

Race, ethnicity, family income, and educational level were determined with a structured interview questionnaire. We used the race and ethnicity categories established by the Census Bureau. The maximum educational level attained by the primary caretaker was categorized as high school or less, any college or technical school, completed college or technical school, any postcollege education, or unknown. Annual household income was elicited in $10 000 increments and categorized as $30 000–$60 000, $60 000–$99 000, and ≥$100 000.

Statistical analyses

All analyses were performed with STATA statistical software (versions 7.0 and 9.0; Stata Corp, College Station, TX). A 2-sided P value of 0.05 was the criterion for statistical significance.

Continuous variables are expressed as means ± SDs or as medians with total and interquartile (25th–75th percentiles) ranges. Categorical variables are expressed as proportions. The relations between $1,25(OH)_2D$, BSAP, and 25(OH)D concentrations were assessed with Spearman's correlation coefficients.

To determine characteristics associated with vitamin D status, a multivariable ordinal logistic regression model was tested with the use of the ologit procedure in STATA to fit proportional odds models (33). Ordinal logistic regression [which considers cutoff values for 25(OH)D] was applied instead of linear regression [which considers actual 25(OH)D concentrations] for several reasons. First, the assumption of linearity for linear regression was not plausible for 25(OH)D concentrations; eg, we did not anticipate that the clinical significance of a 10-point difference in vitamin D concentrations would be the same for 50 compared with 60 ng/mL as for 5 compared with 15 ng/mL concentrations. In addition, the 25(OH)D concentrations violated the assumption of normality for linear regression. We therefore chose to represent 25(OH)D concentrations by using cutoff values that were used in prior studies.

Subjects were grouped into 3 ordinal categories of vitamin D status on the basis of their serum 25(OH)D concentrations: 0 to <20, 20 to <30, and ≥30 ng/mL. To fit the multivariable model, an unadjusted ordinal logistic regression analysis was performed first to examine the association of vitamin D status with demographic, socioeconomic, growth, and dietary characteristics. Factors for which the unadjusted odds ratios (ORs) had an associated P value <0.20 were eligible for inclusion in the multivariable model. Second, we manually fitted a parsimonious multivariable model, using a backward, stepwise strategy, with $P <$ 0.10 as the inclusion criterion. Finally, the goodness of fit of the proportional odds model was assessed by comparing the fitted proportional odds models with multinomial logit models (mlogit, STATA) that do not assume any ordering in the categories of vitamin D status, as suggested in the STATA manual (version 9.0; R, page 345). Multiplicative interaction terms were used to test for interactions between risk factors for hypovitaminosis D. To assess whether the effect of risk factors was the same across vitamin D categories, 2 binary logistic regression models were constructed that separately compared each higher vitamin D category with the lowest category, as additional confirmation of the ordinal logistic regression models.

RESULTS

Subject demographic and growth characteristics

The study enrolled 638 subjects, and vitamin D data were available for 382 subjects. Vitamin D data were not available for all subjects because some of the subjects refused to give blood or an insufficient quantity of serum was obtained for analysis. The subjects for whom vitamin D data were unavailable were significantly younger than those for whom vitamin D data were available (11.3 and 12.4 y; $P < 0.0001$), but the 2 groups did not differ in other demographic (including racial), growth, nutritional, or dietary characteristics or by season of study visit.

The demographic and growth characteristics of the 382 study participants are listed in **Table 1**. The ages of the study participants ranged from 6.0 to 21.6 y. The racial (37% black) and ethnic (7% Hispanic) distribution was typical of the Philadelphia metropolitan area. Forty-four percent of the study visits occurred during the months of November through March (inclusive). The household income and highest level of caregiver education were distributed across a broad spectrum. All the stages of puberty were represented in the sample. The linear growth status was typical of children in the United States, as indicated by the z scores for height. Because of the study screening criteria, the prevalence of obesity was lower than that of the US population (34); 17 children (4.5%) had a BMI z score greater than the 95th percentile. The median total daily vitamin D intake, calculated as the sum of the dietary intake and supplemental intake of vitamin D, was 206 IU (interquartile range: 116-359 IU). Total calcium intake is shown in **Table 2**.

Prevalence of hypovitaminosis D and other serum markers

The median serum concentration of 25(OH)D was 28 ng/mL (interquartile range: 19–35 ng/mL). The 5th and 95th percentiles for the distribution of 25(OH)D concentrations were 10 and 50 ng/mL, respectively. The distribution of subjects across ranges of vitamin D concentrations is shown in **Table 3** and by race and season in **Figure 1**. There were no significant differences in 25(OH)D concentrations between winter and spring and between summer and fall, and thus the 4 seasons were collapsed into 2 broader seasons of wintertime (November through March, inclusive) and summertime (April through October, inclusive). Fifty-five percent of subjects had 25(OH)D concentrations that were inadequate (<30 ng/mL). In children examined during wintertime, the overall prevalence of hypovitaminosis D was 68%; the prevalence was 51% in whites and 94% in blacks. The median intact PTH, available for 322 subjects, was 33 pg/mL (interquartile range: 24-45 pg/mL), and 4% of the subjects had PTH values above the age-specific reference ranges. PTH was shown to be inversely associated with 25(OH)D (Spearman's $r = -0.31$, $P < 0.001$). The distribution of PTH relative to 25(OH)D concentrations is shown in **Figure 2**.

As shown in Table 1, $1,25(OH)_2D$ concentrations were available for 187 subjects: 1 had low concentrations, 67% had normal concentrations, and 33% had concentrations above the upper limit for age. BSAP concentrations were available for 223 subjects: 92% had concentrations in the normal range for age and sex. 25(OH)D concentrations were not correlated with $1,25(OH)_2D$ (Spearman's $r = -0.07$, $P = 0.36$) or BSAP (Spearman's $r = -0.04$, and $P = 0.60$).

TABLE 1
Characteristics of the study sample ($n = 382$)[1]

Characteristic	Value
Age (y)	12.6 (9.9–15.0)[2]
6 to 9 y [*n* (%)]	80 (21)
9 to <12 y [*n* (%)]	84 (22)
12 to <15 y [*n* (%)]	122 (32)
≥15 [*n* (%)]	96 (25)
Male [*n* (%)]	182 (48)
Race [*n* (%)]	
White	206 (54)
Black	141 (37)
Other, unknown, or refused	35 (9)
Ethnicity [*n* (%)]	
Not Hispanic	330 (86)
Hispanic	27 (7)
Unknown	25 (7)
Caregiver annual income [*n* (%)]	
<$30 000	91 (24)
$30 000–$60 000	92 (24)
$60 000–$99 999	80 (21)
≥$100 000	81 (21)
Unknown	38 (10)
Highest caregiver education level [*n* (%)]	
High school or less	87 (23)
Some college or technical school	91 (24)
Graduated from college or technical school	109 (29)
Some postcollege education	93 (24)
Unknown	2 (<1)
Month of study visit [*n* (%)]	
December–February	96 (25)
March–May	104 (27)
June–August	98 (26)
September–November	84 (22)
Weight (kg)	47.9 (31.6–58.9)
Height (cm)	155.8 (137.2–164.7)
BMI (kg/m)	19.2 (16.9–21.9)
Weight *z* score[3]	0.4 (−0.3–0.9)
Height *z* score[3]	0.2 (−0.4–0.7)
BMI *z* score[3]	0.4 (−0.3–1.0)
Lean mass by DXA (kg)	33.6 (23.2–41.8)
Fat mass by DXA (kg)	
<5 [*n* (%)]	89 (23)
5–9.9 [*n* (%)]	132 (35)
10–14.9 [*n* (%)]	93 (24)
≥15 [*n* (%)]	68 (18)
Tanner stage [*n* (%)]	
Stage 1	90 (24)
Stage 2	53 (14)
Stage 3	69 (18)
Stage 4	103 (27)
Stage 5	63 (17)
Unknown	4 (1)
Dietary energy intake (kcal/d)	1817 (1535–2178)
Dietary vitamin D intake (% of AI)[4]	85 (52–121)
Supplemental vitamin D intake (IU/d)	
0 [*n* (%)]	296 (78)
25–200 [*n* (%)]	15 (4)
400 [*n* (%)]	69 (18)
800 [*n* (%)]	2 (<1)
Total daily vitamin D intake (IU)	
< 200 [*n* (%)]	180 (47)
≥200 [*n* (%)]	202 (53)
Intact PTH (pg/mL)[5]	33 (24–45)
Low [*n* (%)]	1 (<1)
Normal [*n* (%)]	307 (95)
High [*n* (%)]	14 (4)
$1,25(OH)_2D$ (pg/mL)[5,6]	62 (51–75)
Low [*n* (%)]	1 (<1)
Normal [*n* (%)]	125 (67)
High [*n* (%)]	61 (33)
Serum calcium (mg/dL)	9.7 (9.4–9.9)
Bone-specific alkaline phosphatase (ng/mL)[5,7]	67 (36–92)
Low [*n* (%)]	6 (3)
Normal [*n* (%)]	205 (92)
High [*n* (%)]	12 (5)

[1] DXA, dual-energy X-ray absorptiometry; AI, adequate intake; PTH, parathyroid hormone; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D.
[2] Median; interquartile range (25th–75th percentiles) in parentheses (all such values).
[3] Data missing for 3 persons.
[4] Data missing for 7 persons.
[5] Age-specific reference ranges given in Subjects and Methods.
[6] Data available for 187 persons.
[7] Data available for 223 persons.

Factors associated with 25(OH)D concentrations

In the unadjusted ordinal logistic regression models, nearly all of the candidate demographic, growth, and nutritional characteristics (described in Table 1) were associated with vitamin D status groups (**Table 4**). Low vitamin D status was more likely to occur in study participants who were older, were black, were members of households with lower annual incomes, were members of households with lower caregiver educational levels, were evaluated during winter months, had greater fat mass, and had higher BMI *z* scores. Low vitamin D status was also associated with lower dietary, supplemental, and total daily intake of vitamin D. Of the candidate variables, only male sex, height *z* score, and daily energy intake were not significantly associated with vitamin D nutritional status ($P > 0.20$).

The interpretation of the ORs presented from the ordinal logistic models in Table 4 and **Table 5** is similar to the interpretation of the ORs in the binary logistic regression. For example, the OR for black subjects (compared with nonblack subjects) is 11.4. This implies that, for black subjects, the odds of being in the <30 rather the ≥30 ng/mL category are 11.4 times the odds for nonblack subjects, given the assumption that all other variables in the model are held constant. Likewise, for blacks, the odds of being in the <20 rather than the ≥20 ng/mL category are 11.4

TABLE 2
Total calcium intake by age group[1]

Age group	$\bar{x} \pm$ SD	Median	Interquartile range[2]
	mg/d		
<9 y ($n = 76$)	766 ± 262	742	587–921
9–12 y ($n = 80$)	812 ± 330	788	533–1030
12–15 y ($n = 123$)	903 ± 494	839	547–1135
≥15 y ($n = 94$)	876 ± 485	761	520–1103

[1] Total calcium intake is dietary calcium plus calcium in supplements.
[2] 25th–75th percentiles.

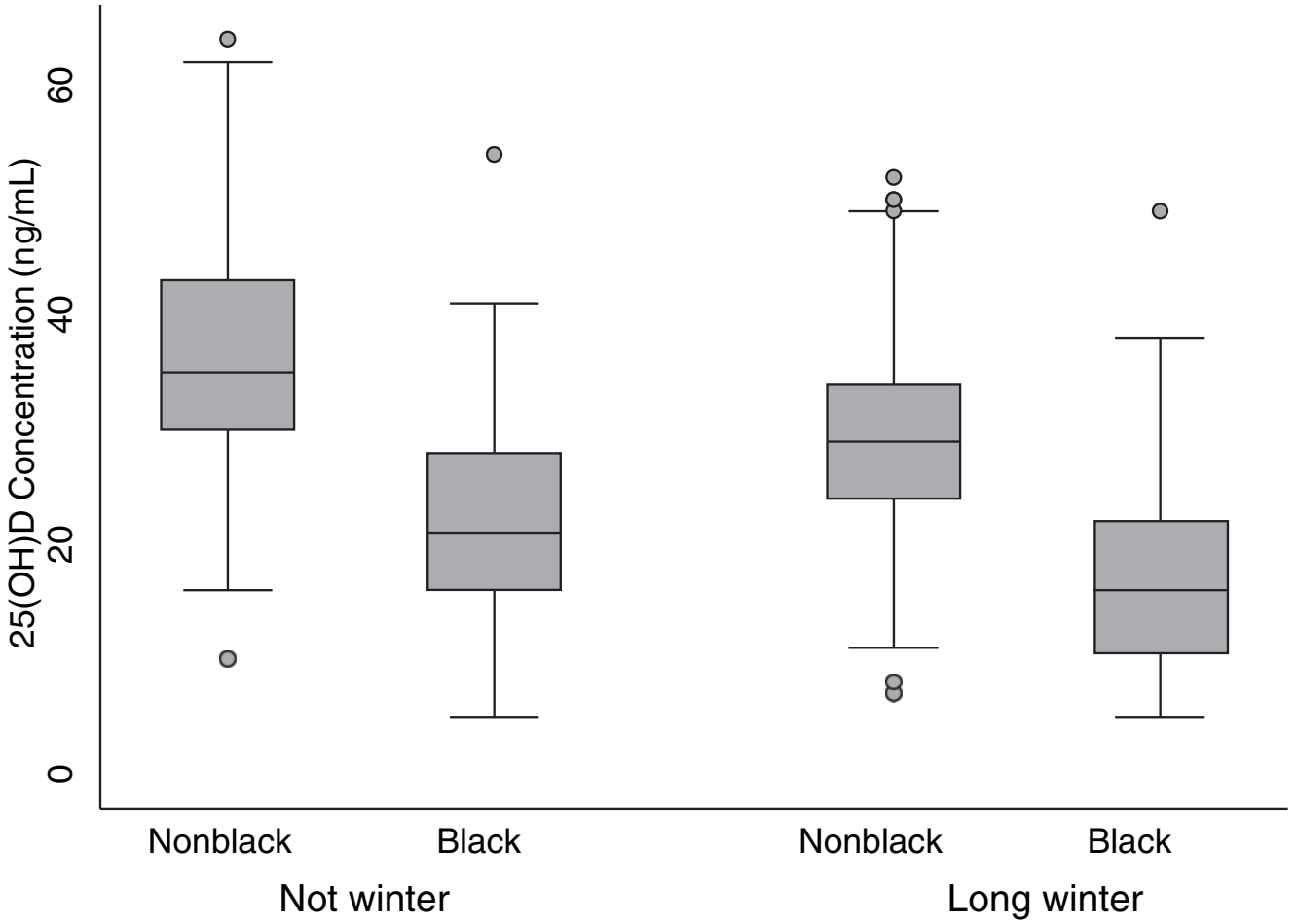

FIGURE 1. 25-Hydroxyvitamin D [25(OH)D] concentrations by race and season were not normally distributed. A 2-factor ANOVA showed significant differences by race ($P < 0.001$) and season ($P < 0.001$) but no significant interaction between race and season.

times the odds for nonblack subjects, given the assumption that all other variables in the model are held constant. The assumption that the odds for comparison of the >30 with the ≤30 ng/mL category are the same as for comparison of the >20 with the ≤20 ng/mL category, known as the proportional odds assumption, was tested and confirmed.

In the multivariable ordinal logistic regression model in which the independent variables are simultaneously adjusted for the other variables in the model, only older age, black race, a study evaluation during winter, and low total daily vitamin D intake were independently associated with decreased vitamin D status (Table 5). Fat mass, lean body mass, caregiver annual income, and other demographic, growth, and nutritional characteristics were not independently associated with vitamin D status. Multiplicative interaction terms between total daily vitamin D intake and a study evaluation during winter ($P = 0.11$) and between race and a study evaluation during winter ($P = 0.79$) were not statistically significant. That is, the associations between vitamin D status and race and between vitamin D status and vitamin D intake did not vary according to season.

TABLE 3
Distribution of serum concentrations of 25-hydroxyvitamin D [25(OH)D]

25(OH)D	Value
	n (%)
<10 ng/mL	18 (5)
10 to <20 ng/mL	79 (21)
20 to <30 ng/mL	112 (29)
≥30 ng/mL	173 (45)

DISCUSSION

Numerous adult studies examined the relations between serum concentrations of 25(OH)D and varied health outcomes and concluded that optimal serum concentrations are >30 ng/mL (17). In this cross-sectional study of otherwise healthy children and adolescents aged 6-21 y, more than one-half of the children had low vitamin D concentrations [25(OH)D <30 ng/mL], and the prevalence was >90% in black subjects during the winter months. In addition, a substantial proportion (19%) of blacks assessed in the winter months had vitamin D concentrations <10 ng/mL, a concentration associated with clinical myopathy, osteomalacia, and rickets (5). In the unadjusted ordinal logistic regression models, numerous factors were associated with decreased vitamin D status. In the adjusted regression model, however, only older age, black race, study evaluation during winter months, and lower total intake of vitamin D were independently associated with decreased vitamin D status. The associations of low vitamin D

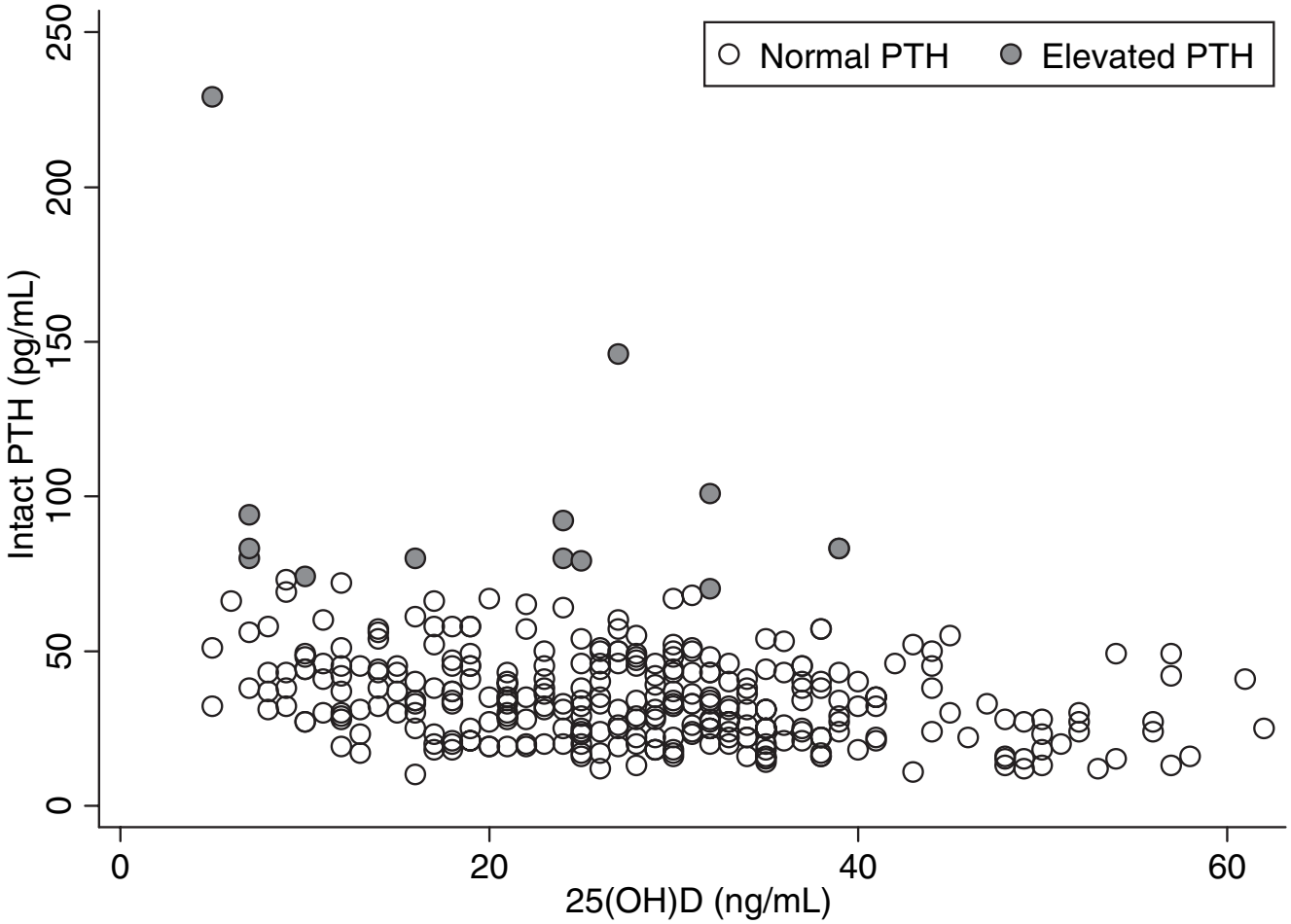

FIGURE 2. Parathyroid hormone (PTH) and 25-hydroxyvitamin D [25(OH)D] concentrations in this sample of healthy children are inversely associated (Spearman's $r = -0.31$, $P < 0.001$). Ranges for elevated PTH are age specific (*see* Subjects and Methods), and PTH concentrations were not elevated in subjects with a 25(OH)D concentration >40 ng/mL.

concentrations with black race and winter season were expected, given the findings from prior studies (11, 22, 35).

In adults, functional measures [net calcium absorption efficiency (36), normal serum PTH concentrations (37, 38), and fracture risk (39)] have been used to define vitamin D adequacy [serum 25(OH)D >30 ng/mL (40)]. Our data show that individuals with elevated PTH concentrations have serum 25(OH)D concentrations approaching 40 ng/mL. Further studies are needed to determine the serum 25(OH)D concentration that indicates vitamin D adequacy for children.

The inverse association of vitamin D status with age during childhood and adolescence has not been well described. Prior studies examined children within a narrow age range, such as adolescence (11–13) or infancy and toddlerhood (14), which limited the ability of these studies to determine whether vitamin D status varied with age. One recent study in girls aged 4-8 y reported an inverse relation between age and 25(OH)D concentrations in blacks but not in whites (35). Previously, our group noted lower vitamin D status with increasing age in children with steroid-sensitive nephrotic syndrome and in the healthy comparison group (22). The age-related decrease in vitamin D status was not attributable to decreased vitamin D intake, because we accounted for vitamin D intake in our multivariable model. Age-related decreases in physical activity (41) likely have an effect on outdoor activities and sun exposure, which represents a plausible explanation for the poorer vitamin D status of older children. Future studies should prospectively measure sun exposure and outdoor activities.

In our multivariable model, lean mass and fat mass adjusted for height were not associated with vitamin D status. Of note, a reported BMI greater than the 95th percentile at the time of the screening phone call was an exclusion criterion, and only 4% of the sample had a BMI in the overweight range. Prior studies in adults reported an association between hypovitaminosis D and obesity (42–44), in which fat mass was measured by whole-body DXA (42, 44) or bioelectrical impedance analysis (43). Obesity has also been shown to be associated with decreased 25(OH)D concentrations in children (11, 16) on the basis of a BMI-based categorization of obesity rather than a direct estimate of fat mass. Other pediatric studies found no association between BMI and 25(OH)D (35). Our lack of association between fat mass and vitamin D concentrations may have been due to the appropriate adjustment for the confounding effects of age, which was significant in our multivariable model. Prior reported associations between fat mass and hypovitaminosis D may have been confounded by the unmeasured effects of sunlight exposure during outdoor activity, because overweight subjects may have less exposure to sunlight and hence have hypovitaminosis D. The contribution of dietary quality and

TABLE 4
Results of unadjusted ordinal logistic regression analyses for associations of subject characteristics with categories of 25-hydroxyvitamin D concentrations[1]

Characteristic[2]	Ordinal odds ratio (95% CI)	*P*
Age (vs <9 y)		0.0001
9 to <12 y	1.72 (0.94, 3.12)	
12 to <15 y	3.30 (1.90, 5.71)	
≥15 y	2.89 (1.62, 5.16)	
Male (vs female)	1.28 (0.88, 1.86)	0.20
Black race (vs nonblack)	11.4 (7.18, 18.0)	< 0.0001
Ethnicity (vs not Hispanic)		0.10
Hispanic	0.43 (0.20, 0.94)	
Unknown ethnicity	0.78 (0.37, 1.67)	
Caregiver annual income (vs <$30 000)		< 0.0001
$30 000–$60 000	0.65 (0.38, 1.10)	
$60 000–$99 999	0.29 (0.16, 0.52)	
≥$100 000	0.17 (0.10, 0.31)	
Unknown	0.47 (0.23, 0.96)	
Highest caregiver education level (vs high school or less)		< 0.0001
Some college or technical school	0.81 (0.47, 1.40)	
Graduated from college or technical school	0.26 (0.15, 0.45)	
Some postcollege education	0.34 (0.20, 0.60)	
Month of study visit (vs December–February)		0.002
March–May	0.63 (0.38, 1.05)	
June–August	0.37 (0.22, 0.63)	
September–November	0.47 (0.27, 0.80)	
Study visit during November through March (vs during other months)	2.73 (1.85, 4.01)	< 0.0001
Weight (kg)	1.03 (1.02, 1.04)	< 0.001
Height (cm)	1.02 (1.01, 1.03)	< 0.001
BMI (kg/m^2)	1.12 (1.06, 1.18)	< 0.001
Weight *z* score	1.25 (1.01, 1.56)	0.04
Height *z* score	1.02 (0.82, 1.26)	0.88
BMI *z* score	1.23 (1.00, 1.50)	0.05
Fat mass by DXA (vs <5 kg)		0.006
5–9.9 kg	1.84 (1.09, 3.10)	
10–14.9 kg	1.90 (1.08, 3.34)	
≥15 kg	2.85 (1.57, 5.16)	
Fat mass-for-height *z* score	1.12 (0.93, 1.35)	0.24
Lean mass-for-height percentile by DXA (vs <25th percentile)		0.24
25th–50th	0.69 (0.40, 1.19)	
50th–75th	0.75 (0.44, 1.28)	
>75th	0.98 (0.59, 1.65)	
Tanner stage (vs stage 1)		< 0.0001
Stage 2	2.18 (1.13, 4.21)	
Stage 3	3.80 (2.03, 7.13)	
Stage 4	3.58 (2.04, 6.28)	
Stage 5	3.56 (1.91, 6.63)	
Dietary energy intake (kcal/d)	1.00 (1.00, 1.00)	0.92
Dietary vitamin D intake (% of AI) (vs 0 to <50%)		0.0002
50 to <75	0.71 (0.39, 1.27)	
75 to <100	0.29 (0.16, 0.55)	
100 to <125	0.44 (0.24, 0.81)	
≥125	0.31 (0.17, 0.55)	
Unknown	0.21 (0.04, 1.21)	
Daily vitamin D supplementation (vs none)		< 0.0001
25–200 IU	0.17 (0.05, 0.63)	
≥400 IU	0.31 (0.18, 0.53)	
Total daily vitamin D intake ≥200 IU (vs <200 IU)	0.38 (0.26, 0.56)	< 0.0001

[1] 25-Hydroxyvitamin D concentrations were categorized into 3 ordinal categories of vitamin D status: 0 to <20, 20 to <30, and ≥30 ng/mL. The sample size for each variable is given in Table 1. AI, Adequate Intake.

[2] The unadjusted ordinal odds ratios were calculated for each variable; the reference group for each variable is given in parentheses. For example, the odds ratio for the age group "12 to <15 y (vs <9 y)" is 3.30, which means that for those aged 12–15 y, the odds of being in the <30 vs the ≥ 30 ng/mL category are 3.3 times the odds of subjects aged <9 y; the odds are the same for the comparison of the <20 vs the ≥20 ng/mL category (on the basis of the confirmed proportional odds assumption).

TABLE 5
Results of multivariable ordinal logistic regression analyses for categories of 25-hydroxyvitamin D concentrations[1]

Variable[2]	Ordinal odds ratio (95% CI)	*P*
Age (vs <9 y)		< 0.0001
9 to <12 y	2.26 (1.13, 4.52)	
12 to <15 y	5.44 (2.85, 10.4)	
≥15 y	5.01 (2.55, 9.83)	
Black race (vs nonblack race)	14.2 (8.53, 23.5)	< 0.0001
Study visit during winter (vs during nonwinter months)	3.55 (2.29, 5.50)	< 0.0001
Total daily vitamin D intake <200 IU (vs ≥200 IU)	1.58 (1.02, 2.46)	0.04

[1] 25-Hydroxyvitamin D concentrations were categorized into 3 ordinal categories of vitamin D status: 0 to <20, 20 to <30, and ≥30 ng/mL. The sample size for each variable is given in Table 1.

[2] The adjusted ordinal odds ratios were calculated with all variables in the table included simultaneously in the model; the reference group for each variable is given in parentheses. For example, the odds ratio for "black race (vs. nonblack race)" is 14.2, which means that for black subjects, the odds of being in the <30 vs the ≥30 ng/mL category are 14.2 times the odds for nonblack subjects, assuming that all other variables in the model are held constant. The odds are the same for the comparison of the <20 vs the ≥20 ng/mL category (on the basis of confirmed proportional odds assumption).

supplement intake to the association of body fat and hypovitaminosis D also remains unknown.

Dietary vitamin D recommendations were established in 1997 (27) and were based on data available to estimate the intake needed to eliminate seasonal variations in PTH concentrations in adults. There were limited data such that an Adequate Intake, rather than a Recommended Dietary Allowance, was established. Many new studies have provided data for adults and children (45). The range of vitamin D required to optimize calcium absorption (36) is better understood, as is the range required to increase serum 25(OH)D concentrations (17, 46–49). In addition, the many non–bone health activities of vitamin D, such as those related to the risk of diabetes, obesity, and some cancers, are now recognized (2). Many children with chronic diseases have suboptimal vitamin D status (22, 50–52), and the current study provides evidence that this is cause for concern in otherwise healthy children. The findings reported here in this multiethnic sample of children and adolescents support the call for a review of the dietary recommendations for vitamin D (45) and underscore the importance of vitamin D intake.

This study has several notable strengths. We examined a large, relatively unselected sample of healthy children. Other studies described vitamin D status in more selected settings, such as in male students in a horseback-riding school (53) or female participants in a gymnastics study (35). Our study included children across a broad age range (6-21 y), to more thoroughly examine age trends. In addition, the sample included children from a broad range of socioeconomic and racial backgrounds and used multivariate statistical techniques to examine the relative contribution of these factors to vitamin D status. 25(OH)D concentrations were measured with the use of ^{125}I-labeled radioimmunoassay, which has excellent results when compared with the gold standard liquid chromatography method (54, 55). Other pediatric studies (14, 56) used a chemiluminescence assay that may have overestimated total 25(OH)D and underestimated 25(OH)D_2 (54, 57). We collected detailed information on intake of vitamin D from dietary and supplemental sources, which showed the important contribution of vitamin D intake to vitamin D status. Also, body fat was measured by DXA, rather than estimating body fat and obesity by using BMI. Finally, multivariable modeling with ordinal logistic regression was used in the analysis. Compared with traditional binary logistic regression, ordinal logistic regression retains the inherent ordinality of 25(OH)D concentrations and avoids arbitrary dichotomization. The categories of vitamin D status spanned a range of modest-to-severe deficits, and the analyses identified a similar effect of risk factors across vitamin D status categories.

This study has several limitations that should also be considered. The sample was conducted at a single site, so our results are not necessarily generalizable to all populations and geographic regions. However, our study sample was large and diverse, consisting of healthy children with few exclusion criteria, so our findings are likely generalizable to many other settings. Vitamin D data were only available for a subset of our full study sample, but selection bias was unlikely because the children who had vitamin D measurements were similar to the children who did not. Finally, sunlight exposure was not measured, but this diverse group of children was probably typical of other healthy-weight urban and suburban children in the mid-Atlantic states. Elucidation of the causes of low vitamin D concentrations in children will require studies that accurately measure dermal synthesis of vitamin D.

In conclusion, low vitamin D status was prevalent in otherwise healthy children in the mid-Atlantic United States. Low serum 25(OH)D concentrations were especially common during the winter months. In addition, older children, black children, and children with low total vitamin D intake were at risk of low circulating 25(OH)D concentrations. These children should be targeted for screening for hypovitaminosis D. Additional studies are needed to document the effects of screening for and treatment of hypovitaminosis D in otherwise healthy children.

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The authors' responsibilities were as follows—all authors: writing of the manuscript; FLW and JS: design and interpretation of the data analysis; and BSZ, MBL, and VAS: overall study design, data collection, and data interpretation. None of the authors had any personal or financial conflicts of interest.

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