

# A prospective analysis of plasma 25-hydroxyvitamin D concentrations in white and black prepubertal females in the southeastern United States<sup>1-3</sup>

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## ABSTRACT

**Background:** Little is known regarding changes in vitamin D status among children living in the southern United States and whether these changes are race-dependent.

**Objectives:** The aims were to prospectively assess plasma 25-hydroxyvitamin D [25(OH)D] concentrations in prepubertal black and white girls ( $n = 83$ ) living in northeast Georgia and to determine whether 25(OH)D concentrations change with increasing age.

**Design:** Plasma samples were obtained annually over a time frame of 1–7 y, and 25(OH)D concentrations were assessed by using radioimmunoassay. Percentage body fat (%BF) and fat-free soft tissue (FFST) mass were measured by using dual-energy X-ray absorptiometry. Linear mixed-effects models were used with height, weight, body mass index percentile, %BF, FFST, pubertal stage, dietary intake, physical activity, and socioeconomic status as covariates.

**Results:** Plasma 25(OH)D values  $<80$  nmol/L were observed in 75% of the participants. Plasma 25(OH)D values (analyzed on the natural logarithm scale) decreased with increasing age ( $P = 0.02$ ), independent of race. Plasma 25(OH)D values were higher in whites than in blacks ( $P < 0.0001$ ), and the amount of this difference depended on season ( $P < 0.001$  for all seasons). A significant negative association between FFST and 25(OH)D, beyond the effects of age, race, and season ( $P = 0.007$ ), was observed. The effects of age, race, and season on 25(OH)D remained significant when dietary calcium, vitamin D, and physical activity were used as covariates; however, after adjustment for FFST, only the effects of race and season remained.

**Conclusions:** White girls living in the southeastern United States have higher 25(OH)D concentrations than do black girls, and the magnitude of this difference depends on the season. Decreases in 25(OH)D with age are associated with increases in FFST. Whether FFST requires additional vitamin D during growth remains to be determined. *Am J Clin Nutr* 2007;85:124–30.

**KEY WORDS** Plasma 25-hydroxyvitamin D, prepubertal females, Georgia, race, season, body composition

## INTRODUCTION

Poor vitamin D status has recently been linked to diseases such as breast (1), colorectal, ovarian, and prostate (2, 3) cancers, cardiovascular disease (4, 5), multiple sclerosis (6, 7), diabetes (8, 9), and the metabolic syndrome (10), as well as to increased

falls and impaired neuromuscular function (11–13). These findings have important implications, because vitamin D insufficiency has been estimated to be present in 20–60% of adults aged  $\geq 50$  y (14). The 2005 Dietary Guidelines Advisory Committee recognized this concern by recommending intakes of 1000 IU vitamin D/d for older adults, persons with darkly pigmented skin, and persons with reduced sun exposure (15).

Little is known about vitamin D status in children, and what is known is based primarily on cross-sectional studies (16, 17). Sullivan et al (18) reported that 48% of girls 9–11 y of age living in Maine (44 °N) had at least one serum measurement below 50 nmol/L over a 3-y period. Although these participants were living at a northern latitude where ultraviolet (UV) B energy during the winter months is inadequate to promote the cutaneous conversion of 7-dehydrocholesterol to previtamin D<sub>3</sub> (19, 20), there is emerging evidence suggesting that persons living at southern latitudes, especially those with darker skin pigmentation, are indeed at risk of low vitamin D status. For example, Levis et al (21) measured serum 25-hydroxyvitamin D [25(OH)D] in adults 18–45 y of age living in Florida (25.46 °N) and reported that 40% of the women studied had serum 25(OH)D concentrations  $<50$  nmol/L at the end of winter (March), whereas at the end of summer (September), 28% of the women had serum 25(OH)D  $<50$  nmol/L and 9% had concentrations  $<30$  nmol/L.

To date, only 2 studies have reported vitamin D status in children and adolescents living at southern latitudes in the United States (16, 17). Looker et al (16) reported that in 12- to 19-y-old females living in southern latitudes (25–41 °N; median: 32 °N) participating in the third National Health and Nutrition Examination Survey (NHANES III; 1988–1994), where blood collection took place in the winter, 12% had serum 25(OH)D  $<37.5$  nmol/L, 29% had values  $<50$  nmol/L, and 47% had values  $<62.5$  nmol/L. Moreover, 70% of the black participants had serum 25(OH)D  $<50$  nmol/L compared with 15% of the white participants. In contrast, data from our laboratory (17), with the use of

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the same 25(OH)D assay (Diasorin) as Looker et al (16), showed that nearly all of the girls studied [4–8 y of age, living in the southern United States (34 °N)] had circulating concentrations of serum 25(OH)D >50 nmol/L, although black girls had significantly lower serum 25(OH)D values than did white girls. The younger age of our participants may partially explain the higher observed circulating 25(OH)D values than those from the 12- to 19-y-old females in NHANES III.

The available studies in children do not address whether vitamin D status changes with growth, nor do they address if the relation between vitamin D and age is dependent on race and season. The purpose of the present study was to prospectively assess plasma 25(OH)D concentrations in prepubertal black and white girls living in northeast Georgia. The study also aimed to 1) determine whether plasma 25(OH)D concentrations change with increasing age; 2) establish whether this pattern is the same between black and white girls, taking into account season; and 3) identify predictors of vitamin D status.

## SUBJECTS AND METHODS

### Study participants

Plasma 25(OH)D samples were collected from girls aged 4–8 y who participated in a prospective University of Georgia Childhood Bone Study (22) in Athens, Georgia (latitude: 34 °N) in the southeast United States. The participants were healthy with no serious medical conditions and did not use medications known to alter bone metabolism. Participant ethnicity (Hispanic, Latino or Non-Hispanic, or Latino) and race (American Indian or Alaska Native, Asian, black or African American, Native Hawaiian or other Pacific Islander, white, or any combination of the above) were classified by parent identification of the participant by using the National Institutes of Health Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research (23). The study sample used data from an intervention trial conducted in our laboratory that investigated the influence of gymnastics on bone (22). Only children in the nonintervention group were included in the present study ( $n = 96$ ). Ethnic and racial groups with small sample sizes ( $n < 7$ ) were excluded from the current project, leaving 83 participants (49 white and 34 black girls) of those originally recruited. Forty-five (27 white, 18 black) participants provided annual plasma samples over 4 y, 16 (14 white, 2 black) over 5 y, 6 (4 white, 2 black) over 6 y, and 1 (white) over 7 y. Before the first testing session, each participant and her guardian completed informed assent and consent forms, respectively. All procedures were approved by the Institutional Review Board for Human Subjects at The University of Georgia.

### Testing protocol

Data collection took place between October 1997 and September 2005. Because of staggered enrollment, plasma samples were obtained throughout the year during winter (December–February), spring (March–May), summer (June–August), and fall (September–November) seasons. Each participant, however, was tested annually within the same season. After the participants fasted overnight, blood was collected between 0730 and 1000 h. Within 1 wk of the blood draw, the participants returned to the laboratory for bone scans, anthropometric measurements, and questionnaires assessing demographic information, dietary intakes, and physical activity.

### Anthropometric measurements

Anthropometric measurements were conducted according to the Anthropometric Standardization Reference protocol (24). The participants were weighed wearing light outdoor clothing without shoes. Participant height and weight were measured to the nearest 0.1 cm and 0.25 kg by using a wall-mounted stadiometer (Novel Products, Rockton, IL) and a calibrated double-beam balance scale (Fairbanks Scales, KS City, MO), respectively. One-way random-effects model and single measure intraclass correlation (ICC) coefficients were computed for anthropometric procedures in girls 6–10 y of age ( $n = 10$ ) who were measured twice in a 2-wk period by the same person in our laboratory. The ICC ( $R$  value) and the test-retest CV (%) values for height and weight were 0.99 and 0.4% and 0.99 and 1.4%, respectively. Body mass index (BMI; in  $\text{kg}/\text{m}^2$ ) values were plotted on BMI-for-age charts (25) to determine BMI percentiles for each child.

### Sexual maturation

Sexual maturation was assessed annually by a physician using criteria for stages of breast development (stages 1–5) as described by Tanner (26). In our laboratory, one-way random-effects model and single measure ICCs revealed perfect agreement ( $R = 1.0$ ) for physician-assessed breast and pubic hair development in girls 6–10 y of age ( $n = 10$ ) from the present study, evaluated by the physician twice in a 2-wk period.

### Plasma 25(OH)D measurement

After an overnight fast, blood samples were collected for analysis of plasma 25(OH)D. Plasma samples were stored at  $-70$  °C until analysis with the use of a radioimmunoassay (DiaSorin Laboratories, Stillwater, MN) and run in duplicate. All samples were assayed by using a block design, such that all samples from the same participant were assayed at one time and an equal number of black and white participants were analyzed with the use of the same kit. The inter- and intraassay CVs were 7.3–10.5% and 5.9–7.0%, respectively.

### Body-composition measurements

Fat-free soft tissue (FFST) mass (in g) and percentage body fat were determined by dual-energy X-ray absorptiometry (DXA; QDR-1000W, Hologic Inc, Waltham, MA). Body-composition measures were analyzed by using DXA Pediatric Whole Body Analysis Software version 5.73. Quality assurance for DXA was performed by daily calibration against the standard phantom provided by the manufacturer. Quality control for soft tissue measurements was assured by concurrently scanning (with each total body scan) an external three-step soft tissue wedge composed of different thickness levels of aluminum and Lucite, calibrated against stearic acid (100% fat) and water (8.6% fat; Hologic Inc). In our laboratory, using a one-way random effects model, we calculated a single measure ICC for percentage fat ( $R = 0.99$ ) for young girls 5–8 y of age ( $n = 10$ ), scanned twice during a 1-wk period. Test-retest measurements by using DXA resulted in a CV of 2.0% for percentage fat.

### Dietary intake

To assess energy (kcal), calcium (mg), and vitamin D ( $\mu\text{g}$ ) intakes, the participants and their parents completed 3-d diet records, a method found to be valid and reliable for estimating

energy and nutrient intakes in children (27–29). To ensure the accuracy of the 3-d diet records, a trained lab technician conducted a 24-h recall using food models and photographs of serving sizes to help train the parents in the accurate recording of food intake. Diet records included time of eating and the type of food and amount, as well as the preparation method. The form specifically inquired about the consumption of calcium-fortified foods and nutritional supplements, and these values were subsequently integrated into the dietary intake data. Because of the prospective analysis of the data, baseline, 12-, and 24-mo diet records were analyzed by using Food Processor II (version 7.5; ESHA Research, Salem, OR) and the 36-, 48-, 60-, 72-, and 84-mo diet records were analyzed by using the Food Processor SQL Nutrition Analysis Software (version 9.7.3; ESHA Research). By using a one-way random-effects model, the ICC for the average measure of 3 d of dietary intake ( $n = 10$ ) completed twice in a 2-wk period was calculated to be  $R = 0.47, 0.71,$  and  $0.94$  for energy, calcium, and vitamin D, respectively, with the use of Food Processor II (version 7.5; ESHA Research) and  $R = 0.86, 0.93,$  and  $0.98$  for energy, calcium, and vitamin D, respectively, with the use of the Food Processor SQL Nutrition Analysis Software (version 9.7.3; ESHA Research). Ten diet records from the first 2 y were analyzed a second time by using the updated software and the results were compared. By using a one-way random-effects model, the ICC for the average measure of 3 d of dietary intake ( $n = 10$ ) completed with the use of Food Processor version 7.5 and again with the use of Food Processor version 9.7.3 was calculated to be  $R = 0.76, 0.90,$  and  $0.99$  for energy, calcium, and vitamin D, respectively.

### Physical activity

Physical activity was quantified subjectively by using a modified version of a questionnaire developed by Slemenda et al (30) that was interviewer-administered to each child and her parent by trained researchers. Using a 5-point Likert scale, the parents indicated their child's usual activity level relative to childhood peers. The questionnaire consists of numerical responses indicating levels of physical activity compared with other children: 1 = much less, 2 = less, 3 = same, 4 = more, and 5 = much more.

### Statistical analyses

Data were analyzed using the Statistical Analysis Software version 9.1 (SAS, Cary, NC). Descriptive statistics were calculated for all variables. A  $P$  value  $\leq 0.05$  was considered statistically significant. Two-tailed independent samples  $t$  tests were used to compare means between black and white girls for all independent variables at baseline.

Linear mixed-effects models were used to analyze the effects of race, age, and season on log-transformed plasma 25(OH)D concentrations. Such models allow for the analysis of between-subject and within-subject sources of variation over time, and are useful when participants enter the study at different ages, when participants are measured repeatedly over time at different ages, or both. The data presented can be considered as providing growth curves for 25(OH)D for 4–12 y old girls of 2 races measured in all 4 seasons. The models used here considered linear and quadratic effects of both baseline age and current age as predictors, thereby allowing for nonlinear trends with age with control for both cross-sectional (between-subject information) and longitudinal (within-subject information) effects of age. In

addition, the models considered main effects of race and season, interactions between these factors (race  $\times$  season), as well as interactions between each of these factors and the linear and quadratic effects of age and baseline age. The model assumed a random subject-specific intercept for each participant to account for between subject heterogeneity, and a residual correlation structure in which the correlation between repeated observations on any given participant is assumed to decrease with the time lag between those measurements. A final model for analysis was developed by first fitting an initial "full" model and then reducing it by dropping nonsignificant main and interaction effects until all retained effects were significant at  $\alpha = 0.05$ . The initial model included the following: 1) linear and quadratic effects of both age and baseline age, 2) main effects of season and race, 3) interactions between race and all age and baseline age effects, and 4) interactions between season and baseline age effects. Age was centered at the mean age (8.53 y) for the entire sample, including baseline to 7 y, and age was used as the metameter for time. Plasma 25(OH)D was analyzed on the natural log scale to correct for nonconstant variance observed on the original scale. Quadratic effects of age were considered here rather than more complex functional relations because scatter plots of the raw data suggested that 25(OH)D and  $\log_e 25(OH)D$  both followed a generally decreasing trend with age with only mild curvature in this pattern. The adequacy of the fitted models was checked using residual plots and other standard diagnostics.

The following covariates were included in the analyses: BMI percentile, breast stage, total calcium intake (including supplements), total vitamin D intake (including supplements), height, physical activity level, body weight, percentage body fat, and socioeconomic status. FFST, a surrogate for growth, was also included as a covariate. To investigate covariate effects, each covariate was added to the model as both a baseline value and as a current value, which allowed for interactions of each with race. Each covariate was added to investigate its relation with  $\log_e$  plasma 25(OH)D concentration. Each covariate was tested for cross-sectional and longitudinal effects as well as for interactions with race. A random subject-specific intercept for each participant was assumed to account for between-subject heterogeneity. Type I sequential  $F$  tests were used to test for the effects of each covariate above and beyond the effects of the variables in the original model (ie, age, race, age<sup>2</sup>, season, and race  $\times$  season). Type 3 marginal  $F$  tests were used to reassess the effects of the original variables after inclusion of (control for) the added covariate.

## RESULTS

### Participant characteristics

Baseline characteristics of the participants are presented in **Table 1**. White and black girls did not differ significantly in age, BMI, body fat percentage, vitamin D intake, or physical activity level; however, significant differences were observed in parent income, FFST, 25(OH)D concentration, and calcium intake (**Table 1**). All but 4 participants were classified as breast stage 1 (ie, prepubertal) with the use of the criteria described by Tanner (26).

### Vitamin D status

At least once during the investigation, 18% and 75% of the participants had plasma 25(OH)D concentrations  $<50$  and

**TABLE 1**  
Baseline descriptive characteristics of the participants

Variable	White girls	Black girls	<i>P</i> for difference <sup>1</sup>	Both races
Age (y)	6.4 ± 1.6 [49] <sup>2</sup>	6.4 ± 1.5 [34]	0.983	6.4 ± 1.6 [83]
Income level <sup>3</sup>	5.2 ± 2.3 [48]	3.2 ± 2.4 [32]	0.001	4.4 ± 2.5 [80]
Anthropometric measures				
BMI (kg/m <sup>2</sup> )	17.4 ± 3.0 [48]	17.8 ± 3.3 [32]	0.526	17.5 ± 3.1 [80]
Percentage body fat (%)	29.4 ± 9.3 [48]	25.2 ± 9.8 [32]	0.057	27.7 ± 9.7 [80]
Fat-free soft tissue mass (kg) <sup>4</sup>	15.8 ± 3.6 [48]	18.1 ± 4.8 [32]	0.018	16.7 ± 4.2 [80]
Physical activity <sup>5</sup>	3.5 ± 0.740 [48]	3.5 ± 0.879 [32]	0.820	3.5 ± 0.794 [80]
Dietary intake <sup>6</sup>				
Vitamin D (μg)	5.6 ± 3.3 [48]	4.5 ± 3.2 [31]	0.130	5.1 ± 3.3 [79]
Calcium (mg)	536.4 ± 149.1 [48]	442.7 ± 148.5 [31]	0.008	499.6 ± 154.9 [79]
Breast stage ( <i>n</i> ) <sup>7</sup>				
Stage 1	48	30		78
Stage 2	0	3		3
Stage 3	0	1		1
25-hydroxyvitamin D (nmol/L)	97.5 ± 21.1 [43]	75.5 ± 22.8 [33]	< 0.0001	87.9 ± 24.3 [76]

<sup>1</sup> Tests of significance between racial groups at baseline are based on 2-tailed independent *t* tests.

<sup>2</sup>  $\bar{x} \pm SD$ ; *n* in brackets (all such values).

<sup>3</sup> Ordinal numbers represent the range of parent income in US dollars (0 = <\$10 000; 1 = \$10 000–\$19 999 . . . 10 = ≥\$100 000+).

<sup>4</sup> Measured by dual-energy X-ray absorptiometry.

<sup>5</sup> Physical activity level was assessed by parent questionnaire developed by Slemenda et al (30), in which 1 = inactive, 2 = below average, 3 = average, 4 = above average, and 5 = very high.

<sup>6</sup> Dietary and supplement intake corrected per 1000 kcal energy intake.

<sup>7</sup> Sexual maturation stage (breast) as described by Tanner (26).

<80 nmol/L, respectively. Five participants (black) had ≥1 plasma 25(OH)D measurement that fell below 37.5 nmol/L, 15 participants (13 black, 2 white) had ≥1 plasma 25(OH)D measurement that fell below 50 nmol/L, and 62 participants (32 black, 30 white) had ≥1 plasma 25(OH)D measurement that fell below 80 nmol/L.

**Race, season, and age**

Statistically significant linear (*P* < 0.001) and quadratic (*P* = 0.020) effects of age were found, showing a decreasing trend that accelerated with age. An interaction between race and season was observed, such that log-transformed plasma 25(OH)D values were higher in the white than black girls, and the magnitude of this difference depended on season (Table 2 and Figure 1). A significant race effect for each season (winter, spring, summer, and fall) was observed such that the difference between the white

and black girls was greatest in winter and least in the spring (Table 2 and Figure 1). No interaction was observed between race and age, indicating that the log<sub>e</sub> 25(OH)D for both whites and blacks decreased in a quadratic pattern over age [ie, the decline in log<sub>e</sub> 25(OH)D did not differ significantly by race]. Plots of the residuals from the fitted model versus age and versus the predicted values showed no pattern, indicating that the effect of age was well described by a quadratic in age and that the log<sub>e</sub> transformation of 25(OH)D was successful in rectifying the nonconstant variance exhibited on the original scale.

**Covariate analysis**

No significant associations were observed between calcium (*P* = 0.919) or vitamin D (*P* = 0.864) intakes and log<sub>e</sub> plasma 25(OH)D. Significant associations were observed between height and FFST and log<sub>e</sub> plasma 25(OH)D above and beyond

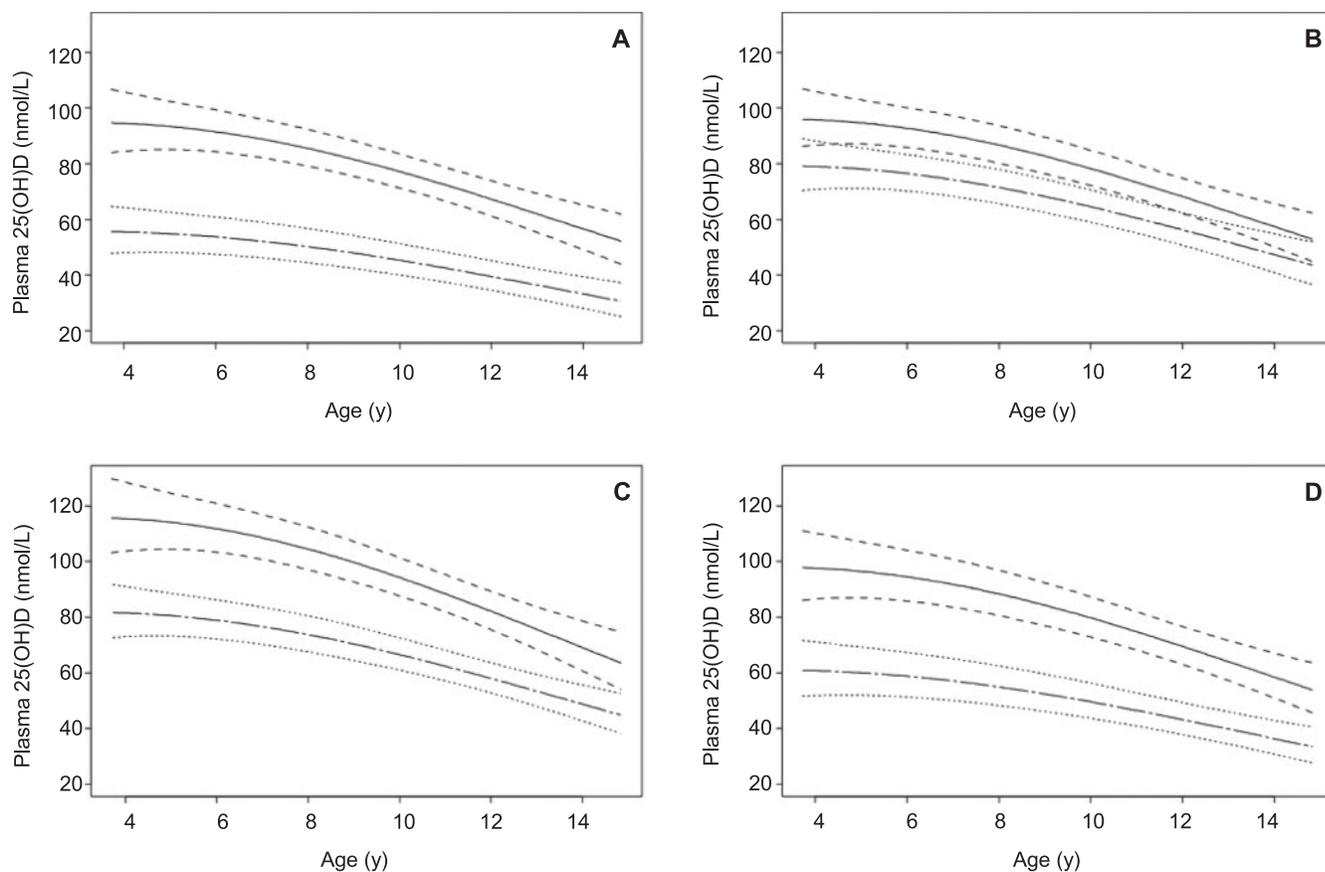
**TABLE 2**  
Estimated mean log<sub>e</sub> plasma 25-hydroxyvitamin D [25(OH)D] and plasma 25(OH)D concentrations by season and race for age<sup>1</sup>

Season	White girls <sup>2</sup>		Black girls <sup>3</sup>	
	Log <sub>e</sub> 25(OH)D	25(OH)D	Log <sub>e</sub> 25(OH)D	25(OH)D
		nmol/L		nmol/L
Winter	4.42 (4.35, 4.50)	83.5 (77.26, 90.19)	3.89 (3.77, 4.01)	49.0 (43.39, 55.41)
Spring	4.44 (4.36, 4.52)	84.6 (78.23, 91.56)	4.25 (4.16, 4.33)	69.8 (64.01, 76.09)
Summer	4.62 (4.55, 4.70)	102.0 (94.81, 109.72)	4.28 (4.19, 4.36)	72.0 (65.98, 78.60)
Fall	4.46 (4.37, 4.55)	86.3 (78.71, 94.57)	3.98 (3.85, 4.11)	53.7 (47.20, 61.02)

<sup>1</sup> All values are  $\bar{x}$ ; approximate 95% CI in parentheses. The mean age of the girls was 8.53 y. The log<sub>e</sub> values are rounded from four decimal places. A significant race × season interaction was observed, *P* < 0.0001 (result is based on a test of the race by season interaction in the linear mixed-model fit to the data). A significant difference in log<sub>e</sub> 25(OH)D across seasons was observed for white and for black girls, *P* < 0.0001 for both (results are based on tests of contrasts in the race by season means from the linear mixed-model fit to the data).

<sup>2</sup> Annual plasma samples were provided by 27, 14, 4, and 1 girls over 4, 5, 6, and 7 y, respectively.

<sup>3</sup> Annual plasma samples were provided by 18, 2, and 2 girls over 4, 5, and 6 y, respectively. No girls provided annual plasma samples over 7 y.



**FIGURE 1.** Plasma 25-hydroxyvitamin D [25(OH)D] by age and race in winter (A), spring (B), summer (C), and fall (D). Results of linear mixed-effects model describing relations between  $\log_e$  25(OH)D and age, race, and season. Plots display back-transformed fitted values on the original 25(OH)D scale. —, mean plasma 25(OH)D for the white girls; - - -, mean plasma 25(OH)D for the black girls; - - - - -, pointwise 95% CI for the white girls; ·····, pointwise 95% CI for the black girls. All significant results are based on hypothesis tests on parameters of the fitted model (eg, hypothesis tests on regression coefficients and contrasts). The quadratic and linear terms in the fitted relation between age and  $\log_e$  25(OH)D are significant ( $P = 0.0201$  and  $P < 0.0001$ , respectively). Significant differences in  $\log_e$  25(OH)D between the black and white girls are found in winter ( $n = 7$  and 14, respectively;  $P < 0.0001$ ), spring ( $n = 16$  and 17, respectively;  $P = 0.0009$ ), summer ( $n = 10$  and 12, respectively;  $P < 0.0001$ ), and fall ( $n = 1$  and 6, respectively;  $P < 0.0001$ ).

the effects of age,  $\text{age}^2$ , race, and season ( $P = 0.025$  and  $0.007$ , respectively). Furthermore, the association of weight and  $\log_e$  plasma 25(OH)D above and beyond the effects of age,  $\text{age}^2$ , race, and season was marginally significant ( $P = 0.053$ ). When using dietary calcium and vitamin D as covariates, the significant effects of age, race, and season on  $\log_e$  plasma 25(OH)D remained. After control for height and weight, only age and season remained statistically significant. Race and season, but not age, remained significant after control for FFST.

## DISCUSSION

The main finding of the present prospective investigation was that vitamin D status declined with age for both black and white prepubertal girls aged 4–8 y at baseline living in the southeastern United States. After adjustment for FFST, there were no longer declines in circulating plasma 25(OH)D with age, implying an important relation of 25(OH)D and lean mass with growth. Black participants had lower plasma 25(OH)D concentrations throughout the study than did the white participants, and the differences between the white and black girls depended on the season of blood collection. Despite living in the southeastern United

States, 18% of the participants had  $\geq 1$  plasma 25(OH)D measurement that fell below 50 nmol/L, and 75% had  $\geq 1$  measurement that fell below 80 nmol/L.

The present study is the first to show an age-related decline in circulating 25(OH)D concentrations for both black and white girls. All prior studies investigating vitamin D status in children or adolescents have been cross-sectional (16, 17, 21) or quasi-cross-sectional (18). Although not the primary outcome of a vitamin D supplementation trial in Finnish adolescents, serum 25(OH)D declined 20% in the placebo group over the course of the 2-y study (31). The explanation for the declining 25(OH)D values is unknown but may be related to UVB exposure, dietary intakes of calcium and vitamin D, increased nutrient needs during growth, or changes in physical activity. One limitation of the present study was that we did not measure UVB exposure or time spent outdoors, so it is unknown whether UVB exposure also declined with age and contributed to the declining circulating 25(OH)D concentrations. At baseline, the mean intake of calcium was  $\approx 300$  mg below the recommended 800 mg/d for children, and the mean intake of vitamin D was equivalent to the recommended 5  $\mu\text{g}$  vitamin D/d (32). Control for the change in vitamin D and calcium intakes along with physical activity did

not alter the significant relation between vitamin D status and age, showing that these variables did not influence the age-related changes in vitamin D. It was shown in adults that the current dietary recommendations for vitamin D are inadequate to maintain 25(OH)D concentrations in the absence of substantial cutaneous vitamin D production (33). Our data also imply that meeting the recommended 5  $\mu\text{g}$  vitamin D/d does not appear to be sufficient to maintain circulating vitamin D concentrations in young children.

The decline in vitamin D status with age was eliminated after control for FFST mass, suggesting that increases in lean mass with growth are associated with a decline in circulating concentrations of 25(OH)D. Our results are consistent with those reported by Jones et al (34) in a population of 8-y-old Tasmanian children, where a negative correlation between log-transformed 25(OH)D with lean mass, calculated from skinfold-thickness measurements, was reported. In contrast, El-Hajj Fuleihan et al (35) found that Lebanese premenarcheal females supplemented with either 1400 IU/wk or 14 000 IU/wk vitamin D<sub>3</sub> for 1 y had significant increases in FFST compared with those who received placebo. When lean mass was used as a covariate in their regression analyses, the relations between vitamin D and BMC weakened, highlighting the potential role of lean mass in mediating the effects of vitamin D on bone (35). Another 2-y vitamin D<sub>3</sub> supplementation trial conducted in Finnish adolescent females at a level of 200 IU/d showed no increase in FFST (31), but the discrepancies between the findings of these 2 studies may be due to geographical differences between Lebanon (33.5 °N) and Finland (62 °N), genetic differences between Lebanese and Finnish children, and differences in baseline characteristics of the participants, such as vitamin D intakes, baseline 25(OH)D concentrations, and differences in pubertal stage. Most of the participants in the Finnish study were in sexual maturation stage 1 and may not have experienced significant muscle growth during the 2-y intervention because peak growth velocity for lean tissue mass occurs  $\approx$ 13.5 mo before menarche (31).

Muscle spasms, weakness, and atrophy are present in clinical vitamin D deficiency (36, 37). Calcitriol (1,25-dihydroxyvitamin D) stimulates the growth and proliferation of muscle cells (myogenesis) and regulates intracellular calcium in muscle cells (contractility) (36). Circulating calcidiol and calcitriol concentrations have been positively associated with muscle strength and function in older adults (38–40), possibly due to the direct interaction between 1,25-dihydroxyvitamin D and its nuclear receptor located in muscle cells (41). In our sample, the girls who acquired greater lean mass (represented by FFST) throughout growth displayed greater decreases in plasma 25(OH)D concentrations. It is plausible that the increase in FFST with age leads to increased vitamin D use by growing muscle and a decrease in circulating 25(OH)D.

The black participants had lower plasma 25(OH)D concentrations throughout the study than did the white participants, which is consistent with previous studies conducted in adolescents (16, 42–44) and younger children (17, 45). We unexpectedly found a race-by-season interaction, such that the difference between plasma 25(OH)D concentrations in black and white girls depended on the season of blood collection. The difference between the white and black girls was greatest during the winter and fall and smallest during the spring and summer. Only one study reported an interaction between season and race in a sample of 20- to 40-y-old black and white women living in Boston, where

there was a significant time-by-race interaction for the 4 time points (ie, spring, summer, fall, and winter), suggesting that the seasonal variation in 25(OH)D is smaller for black women than for white women (46). In the present study, the black girls had a greater seasonal variation in plasma 25(OH)D than did the white girls. Plasma 25(OH)D increased dramatically from winter to spring in the black girls, which may have been due to the spring break from school during which vacations or more time spent outdoors may have affected circulating vitamin D. Neither vacations nor sunscreen use was documented in the present study. Another limitation of our study was that samples were collected only once annually for each participant, and therefore within-subject seasonal variation could not be assessed.

The present study is the first to report potentially compromised vitamin D status in children <12 y of age living in the southern United States, where UVB radiation is assumed to be adequate to prevent low vitamin D status. In our previous cross-sectional investigation, we found that nearly all participants in a sample of 4- to 8-y-old girls had serum vitamin D concentrations >50 nmol/L (17). In the present study, we showed that, although this group of girls had plasma vitamin D concentrations >50 nmol/L at baseline, 25(OH)D concentrations declined with age, and a considerable number of girls had vitamin D concentrations that were <50 nmol/L. The present study further supports the notion that living at lower latitudes is not necessarily protective against vitamin D insufficiency (47).

In conclusion, circulating concentrations of 25(OH)D declined with growth, and this may be related to the increased utilization of vitamin D by soft tissue. In this sample of young females living in the southeastern United States, 18% had 25(OH)D concentrations that fell below 50 nmol/L at least once during the investigation. Further investigation into the utilization of 1,25-dihydroxyvitamin D by muscle and the subsequent effect on circulating concentrations of 25(OH)D in children may help elucidate the relation between FFST mass and vitamin D status. Such advances in the field of vitamin D nutrition may help to answer the question of whether more vitamin D is required during this period of growth. 

RDL, EML, DBHau, DBHal, and CMW were responsible for the study concept and design. RDL, EML, and CMW were responsible for the acquisition of the data. DBHau and CMW were responsible for the plasma 25(OH)D analysis. DBHal conducted the statistical analyses. RDL, EML, DBHau, and CMW were responsible for the interpretation of the data and drafting the manuscript. All authors contributed to the revision of the manuscript. None of the authors had any personal or financial conflicts of interest.

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