Vitamin D Status and Calcium Metabolism in Adolescent Black and White Girls on a Range of Controlled Calcium Intakes

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Background: There are limited data in adolescents on racial differences in relationships between dietary calcium intake, absorption, and retention and serum levels of calcium-regulating hormones.

Objectives: The aim of this study was to investigate these relationships cross-sectionally in American White and Black adolescent girls.

Methods: Calcium balance studies were conducted in 105 girls, aged 11–15 yr, on daily calcium intakes ranging from 760–2195 mg for 3-wk controlled feeding periods; 158 observations from 52 Black and 53 White girls were analyzed.

Results: Black girls had lower serum 25-hydroxyvitamin D [25(OH)D], higher serum 1,25-dihydroxyvitamin D, and higher calcium absorption and retention than White girls. Calcium intake and race, but not serum 25(OH)D, predicted net calcium absorption and retention with Black girls absorbing calcium more efficiently at low calcium intakes than White girls. The relationship between serum 25(OH)D and serum PTH was negative only in White girls. Calcium intake, race, and postmenarcheal age explained 21% of the variation in calcium retention, and serum 25(OH)D did not contribute further to the variance.

Conclusions: These results suggest that serum 25(OH)D does not contribute to the racial differences in calcium absorption and retention during puberty. **(*J Clin Endocrinol Metab* 93: 3907–3914, 2008)**

Adequate calcium nutrition plays a major role in the development and maintenance of peak bone mass during childhood and adolescence and the subsequent prevention of osteoporosis (1). Calcium absorption and bone calcium accretion are affected by dietary calcium and vitamin D status. The former can be assessed by diet history and the latter by serum concentration of 25-hydroxyvitamin D [25(OH)D]. Serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] on the other hand does not assess vitamin D status but, to a large extent, the hormonal status of the serum calcium-regulating system. An inverse relationship between serum 25(OH)D and PTH has been reported in children (2–5), suggesting that vitamin D status is insufficient in some children The impact of vitamin D status on skeletal calcium accretion in children on controlled calcium intakes is unknown.

American Black adolescents have higher calcium absorption and bone accretion than White adolescents (6). American Black children have higher bone mass than American White children (7, 8) Despite higher bone mass and calcium utilization, vitamin D status is typically lower in Black children (9). Serum 25(OH)D levels of less than 15 ng/ml were reported in 24% of 307 healthy Bostonian children, with the highest prevalence of vitamin D deficiency in Black teenagers during winter (4). In a prospective study of 83 Black and White girls from the State of Georgia, age 4–8 yr, the greatest difference in vitamin D status between races

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2008-0645 Received March 20, 2008. Accepted July 29, 2008.

First Published Online August 5, 2008

Abbreviations: BMC, Bone mineral content; Cr, creatinine; CV, coefficient of variation; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PEG, polyethylene glycol; PMA, postmenarcheal age; NTx, cross-linked N-telopeptides of type 1 collagen.

was at the end of the summer months, reflecting the greater seasonal fluctuation in White compared with Black girls (10).

We have reported the impact of calcium intake and race on skeletal calcium accretion in adolescent girls elsewhere (11). Adolescent Black compared with White girls on similar calcium intakes have higher skeletal calcium retention and bone formation rates determined by calcium kinetics despite similar biochemical markers of bone turnover (6, 11). The gold standard for measuring bone turnover rates is calcium kinetics (12). However, the effect of vitamin D status on these relationships is unknown. Thus, we explored the interrelationships of serum 25(OH)D, $1,25(OH)_2D$, PTH, and bone turnover on calcium absorption, skeletal calcium retention, and bone mass in American White and Black adolescent girls over a wide range of controlled dietary calcium intakes. Cross-sectional data from adolescent calcium balance studies using the same protocol were pooled to investigate these relationships using a large sample size.

Subjects and Methods

Subjects

Healthy adolescent girls were recruited from middle schools between 1990 and 2000 as previously described (6, 11, 13–15). Several subjects participated in two or more study periods yielding a total of 158 observations from 52 Black and 53 White girls. For most subjects, the repeat studies occurred in the same summer while participating in a study on calcium intake that was a crossover design. The children had no history of bone disease and had not taken medications known to affect bone. General health was determined by medical history questionnaire, physical examination, and blood biochemistry. Subjects were matched for sexual maturity by using questionnaires administered by a research coordinator to determine postmenarcheal age (PMA) and Tanner stage (average of breast and pubic) (16) at the time of study. To characterize sexual maturity of the subjects, PMA included negative values for girls who had not yet begun menses at the time of the study; exact time of menarche was determined through follow-up contacts. Race was established by race of parent and grandparents.

Subjects lived at Purdue University in campus housing and participated in 3-wk metabolic studies structured as summer camps. All the blood samples were drawn during the summer months in Indiana (37–41 degrees latitude). The children did not use sun screens and spent about 30% of the day in outdoor activities including swimming and softball. Protocols were consistent over the duration of the studies. The studies were approved by the Purdue University and Indiana University School of Medicine and Clarian Institutional Review Boards. Subjects gave written assent, and guardians gave their written consent for participation.

Anthropometrics and bone measurements

Total body bone mineral content (BMC) was measured by dual-energy x-ray absorptiometry (GE Lunar, Madison, WI) during the balance period. Short-term precision is 1.68% for total body BMC. Standing height using a wall-mounted stadiometer and weight using an electronic digital scale were measured on lightly clad subjects without shoes at the time of the dual-energy x-ray absorptiometry scan.

Dietary intake analysis

Dietary intake was controlled, and subjects ingested only foods prepared by staff. A 4-d cycle menu was developed, and a composite of meals for each day was saved for analysis. All food and beverages were prepared with deionized water and weighed to the nearest 0.1 g. Calcium intake was varied above the basal diet through fortified sources to achieve intakes ranging from 760-2195 mg calcium/d. After consuming calcium-rich beverages such as milk and calcium-fortified juice and foods such as ice cream, subjects rinsed the containers with deionized water, and the rinse was also consumed. Discretionary salt was not allowed. Any food not consumed before the end of the day was collected and weighed. A daily meal composite was frozen for analysis of mineral content. Diet composites and any leftover portions were thawed, homogenized, and freeze dried (FTS Systems, Inc., Stone Ridge, NY) for later analysis.

Sample collection and analysis

Subjects collected all urine and feces in acid-washed containers that were pooled as 24-h collections. Diet and fecal samples were prepared similarly. Diet, fecal, and urine samples were measured for calcium content in triplicate using National Bureau of Standards reference standards for monitoring quality control as previously described (6, 11, 13–15).

Completeness of collections

Creatinine (Cr) was used both to assess completeness of urine collections based on a constant daily urinary Cr excretion and to adjust for variation in timing of 24-h collections. Urinary Cr was measured on unacidified urine using a colorimetric procedure on a Cobas Mira Plus (Roche Diagnostic Systems, Branchburg, NJ). Daily urine samples that fell less than 11 mg creatinine/kg body weight were omitted from the analysis.

Polyethylene glycol (PEG) (Dow Chemical Co., Midland, MI) was used for assessing fecal collection completeness. Each subject ingested 3 g PEG/d in divided doses of 1 g/meal. PEG was analyzed in triplicate using a turbidimetric assay (17). Recovery of PEG was used to exclude subjects' data if compliance was poor. Subjects included in this analysis represent those already screened for compliance in the original studies (6, 11, 13–15).

Retention and absorption calculations

Calcium balance or retention was calculated by subtracting calcium excretion through urine and feces from dietary calcium intake. The first week of each 3-wk study was regarded as an equilibration period, and the last 14 d served as the experimental period. Balance was calculated for as many days within this 14-d period as met the requirement of beginning and ending with a fecal sample. Net calcium absorption efficiency was calculated as intake minus fecal excretion divided by intake $\times$ 100.

Hormone and biomarkers of bone metabolism

Blood was drawn after an 8-h overnight fast near the end of each metabolic study. Serum intact PTH was measured by a two-site immunoassay [coefficient of variation (CV) of 9.7% at 17.5 pg/ml] (Nichols Institute Diagnostics, San Juan Capistrano, CA). Serum 25(OH)D and $1,25(OH)_2D$ were analyzed by RIA (CV of 8.1 and 9.1%, respectively) (DiaSorin, Stillwater, MN). Serum osteocalcin was analyzed by RIA (CV of 8.9% at 26.9 ng/ml) (Nichols Institute). Serum bone alkaline phosphatase was measured by ELISA (CV of 4.1%) (Quidel, San Diego, CA). Urine cross-linked N-telopeptides of type 1 collagen (NTx) was measured by an ELISA (CV of 7.5%) (Osteomark; Ostex International, Inc., Seattle WA).

Statistical analysis

Statistical analysis was performed using SAS (version 9.1; SAS Institute, Cary, NC). Subject characteristics were compared using *t* tests. Two-way ANOVA with Bonferroni corrections was used to determine effect of race and calcium intake on balance variables. Models were developed to study the relationships between calcium absorption or retention and race, calcium intake, PMA, serum 25(OH)D, $1,25(OH)_2D$, PTH, and biochemical markers of bone turnover including osteocalcin, bone alkaline phosphatase, and urine NTx/Cr. These variables are those generally considered to be involved in calcium absorption and retention. Calcium intake, race, and PMA were the primary determinants examined, but other covariates and their necessary interactions were evaluated in the context of this model. Because diagnostic plots indicate that relationships with PMA are best modeled with negative values of PMA recoded to zero, all analysis with PMA were performed with recoded

TABLE 1. Characteristics of Black and White girls

	Racial difference	Mean ± SD (min–max)	
		Black (n = 52)	White (n = 53)
Age (yr)	Black < White[a]	12.1 ± 1.1 (10.0–14.5)	12.8 ± 1.1 (11.0–15.1)
Height (cm)		157.9 ± 7.9 (141.0–173.4)	157.9 ± 6.4 (146.0–177.0)
Weight (kg)		54.1 ± 12.2 (32.0–84.3)	54.3 ± 13.4 (37.0–95.0)
BMI (kg/m^2)		21.6 ± 3.8 (15.6–29.3)	21.3 ± 4.4 (15.4–36.2)
PMA (months)		10.5 ± 12.8 (−0.6–44.0)	7.1 ± 12.7 (−20.0–36.0)
Tanner stage (average)		3.8 ± 0.9 (1–5)	3.7 ± 0.8 (2–5)
Total body BMC (g)	Black > White[b]	2227 ± 502 (1278–3479)	2051 ± 389 (1427–3172)
Lean body mass (kg)		37.5 ± 5.41 (25.04–51.29)	35.5 ± 4.49 (26.58–50.03)
Serum calcium (mg/dl)		9.3 ± 0.5 (8.6–10.6)	9.5 ± 0.4 (8.6–10.5)
Serum 25(OH)D (ng/ml)	Black < White[a]	25.7 ± 7.8 (11.8–51.4)	33.2 ± 10.4 (13.9–71.8)
Serum 1,25(OH)D_2 (pg/ml)	Black > White[a]	45.4 ± 13.4 (11.2–78.6)	36.0 ± 8.1 (14.8–49.4)
Serum PTH (pg/ml)		28.0 ± 11.9 (9.9–57.9)	24.9 ± 9.4 (6.7–60.3)
Urine NTx/Cr (nmol BCE/mmol Cr)		380 ± 224 (71–1118)	465 ± 255 39–1187
Serum osteocalcin (ng/ml)		38.3 ± 27.1(5.6–133.3)	45.7 ± 27.9 (0.0–147.0)
Serum bone alkaline phosphatase (ng/ml)		69.8 ± 33.6 (21.0–184.8)	72.1 ± 40.6 (5.8–198.5)

BCE, Bone collagen equivalents; BMI, body mass index.

[a] $P < 0.01$.

[b] $P < 0.05$.

values. The effect of year of study on residuals was evaluated to ensure that there was no influence of year of study on the relationships. Statistical significance was assessed using the bootstrap method (18).

Results

Subject characteristics (Table 1)

Black girls were chronologically younger but had similar sexual maturity (PMA, Tanner stage) compared with White girls. Black girls had higher total body BMC than White girls. Black girls had lower serum 25(OH)D ($P < 0.0001$) (Fig. 1) and higher serum 1,25$(OH)_2$D ($P = 0.0003$) than White girls. Height, weight, body mass index, total lean body mass, biochemical markers of bone turnover, and PTH were not different between Black and White girls.

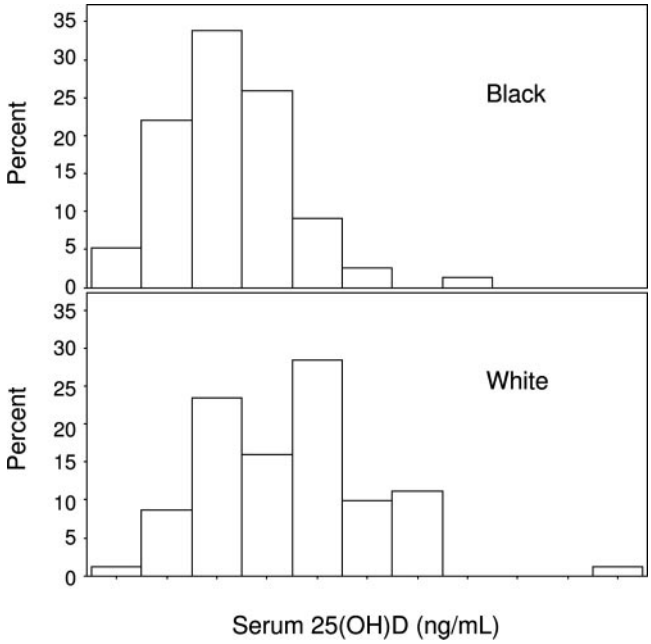

FIG. 1. Distribution of serum 25(OH)D in 52 Black and 53 White adolescent girls in summer. For nanomoles per liter, multiply by 2.496. Mean difference due to race was significant at $P < 0.0001$.

Effect of race and calcium intake on calcium balance variables (Table 2)

Black girls had lower urinary calcium ($P < 0.01$) and fecal calcium ($P < 0.01$) and higher net calcium absorption ($P < 0.01$) and calcium retention ($P < 0.01$) than White girls. Fecal calcium excretion, net calcium absorption, and calcium retention were lower on a low calcium intake ($P < 0.01$) than on a high calcium intake for both races.

Relationship between serum 25(OH)D and calcium absorption and retention (Figs. 2 and 3)

Neither calcium absorption nor retention were related to serum 25(OH)D (Fig. 2). There was a significant relationship between serum 25(OH)D and PTH, but only in White girls (Fig. 3). For White girls, an increase of 1 ng/ml serum 25(OH)D was associated with a decrease of 0.33 pg/ml serum PTH ($P = 0.0007$). For Blacks, there was no relationship ($P = 0.30$).

Relationship between serum 1,25$(OH)_2$D and calcium absorption and retention (Fig. 4)

Neither calcium absorption nor retention were related to serum 1,25$(OH)_2$D (Fig. 4). Serum 1,25$(OH)_2$D was not related to serum PTH.

Prediction of calcium balance variables (Table 3)

Calcium retention

The best model for calcium retention included race, calcium intake, and urinary NTX/Cr ($r^2 = 0.29$; $P < 0.01$). The addition of serum 25(OH)D or 1,25$(OH)_2$D was not significant in the model.

TABLE 2. Calcium balance variables in relation to race and calcium intake

	Mean ± SD (min–max)	
	Black (n = 52)	**White (n = 53)**
Calcium retention (mg/24 h); L < H[a]; Black > White[a]		
L	455 ± 135 (216–724)	256 ± 151 (−60–559)
M	488 ± 206 (103–913)	441 ± 242 (153–1007)
H	666 ± 417 (10–1583)	459 ± 267 (56–1179)
Urinary calcium (mg/24 h); Black < White[a]		
L	48 ± 39 (1–173)	82 ± 54 (26–181)
M	40 ± 29 (7–100)	90 ± 50 (24–200)
H	76 ± 63 (3–182)	106 ± 47 (29–192)
Fecal calcium (mg/24 h); L < M < H [a]; Black < White[a]		
L	304 ± 121 (63–581)	486 ± 168 (154–719)
M	758 ± 230 (277–1218)	817 ± 201 (319–1053)
H	1172 ± 369 (447–1867)	1331 ± 281 (703–1949)
Calcium absorption, efficiency (%); Black/L > all others		
L	62.3 ± 15.0 (30.4–92.3)	41.0 ± 20.2 (13.0–80.7)
M	41.4 ± 16.3 (10.1–77.1)	39.1 ± 15.7 (19.6–76.7)
H	38.6 ± 19.4 (3.3–78.0)	29.9 ± 13.5 (11.2–63.6)
Net calcium absorption (mg/24 h); L < H [a]; Black > White[b]		
L	503 ± 125 (253–758)	337 ± 163 (108–644)
M	527 ± 197 (122–933)	531 ± 223 (256–1049)
H	743 ± 395 (64–1586)	565 ± 258 (210–1228)

Low calcium intake (L) is 787–854 mg; medium calcium intake (M) is 1205–1491 mg; high calcium intake (H) is 1795–2195 mg. For Black subjects, n = 22 for low, 21 for medium, and 9 for high calcium intake; for White subjects, n = 16 for low, 21 for medium, and 16 for high calcium intake.

[a] $P < 0.01$.

[b] $P < 0.05$.

Urine calcium

A model with race, calcium intake, PMA, and urinary NTx/Cr explained 30% ($P < 0.01$) of the variation in urinary calcium. The addition of serum 25(OH)D or $1,25(OH)_2D$ was not significant in the model.

Fecal calcium

A model with race and calcium intake explained 69% ($P < 0.01$) of the variation in fecal calcium. Addition of urinary NTx/Cr improved the prediction by 2% ($P < 0.01$). The addition of serum 25(OH)D or $1,25(OH)_2D$ was not significant in the model.

Net calcium absorption

Race and calcium intake explained 19% ($P < 0.01$) of the variation in net calcium absorption. Adding PMA improved the prediction to 20% ($P < 0.01$). The addition of serum 25(OH)D or $1,25(OH)_2D$ was not significant in the model.

Total body BMC

Total body BMC was not predicted by race. PMA predicted 22% of the variation, and lean mass explained an additional approximately 50% of the variation in total body BMC. The addition of serum 25(OH)D or $1,25(OH)_2D$ was not significant in the model.

Discussion

In this study, we pooled data from multiple metabolic calcium balance studies using the same protocol on a wide range of calcium intakes, to investigate, in a sample of large size, the role of vitamin D status on skeletal calcium accretion and absorption in White and Black adolescent girls. The distribution of serum 25(OH)D was wide with a range of 12–51 ng/ml in Blacks and 14–72 ng/ml in Whites, even though all the blood samples were drawn during the summer months in Indiana (37–41 degrees latitude). Serum 25(OH)D was lower in Black than White girls as has been shown in other studies (4, 9, 10, 19) with 82% Blacks and 47% Whites in the insufficiency range using a level of less than 30 ng/ml as the cutoff point in adults (20). The distribution in vitamin D status was similar to that of a study in 93 adolescent boys and girls from Houston, TX (5), but higher than that found in 370 adolescents in a year-round study from Boston, MA (4). Calcium retention was not influenced by vitamin D status in White or in Black adolescent girls. These are the only published studies on the effect of vitamin D status on skeletal calcium retention in children measured over a 3-wk calcium balance period. Our studies demonstrate that vitamin D insufficiency had no adverse effects on calcium retention. Indeed, Blacks who had lower vitamin D status than Whites had the higher calcium retention. Furthermore, no relationship was found between vitamin D status and BMC measured at the time of balance, which supports our balance results and a study performed in 93 adolescent boys and girls studied in Texas (5). The main factors determining BMC in our study were lean body mass and pubertal maturation. Vitamin D status did not predict BMC. The significant ($P = 0.049$) racial difference in BMC observed in Table 1 is not significant in a model that includes PMA shown in Table 3 ($P = 0.065$).

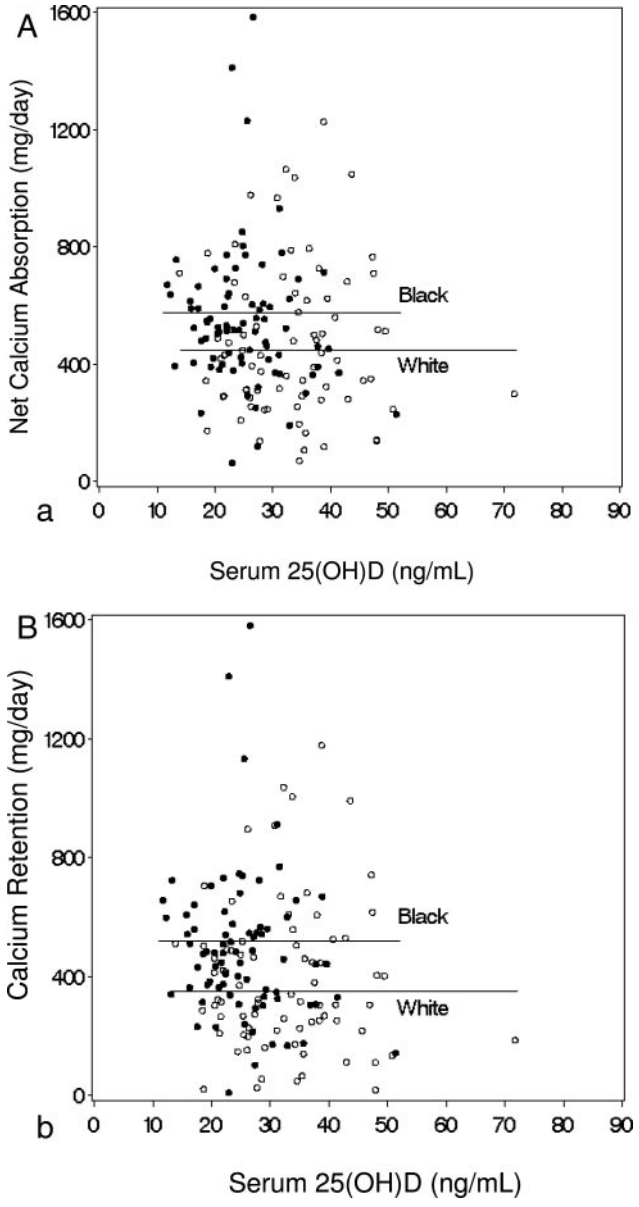

FIG. 2. Relationship of serum 25(OH)D and net calcium absorption (A) and net calcium retention (B) in adolescent girls. Controlling for daily calcium intake, Blacks had higher net calcium absorption ($P = 0.003$) and higher net calcium retention ($P = 0.003$) than White girls. Serum 25(OH)D did not predict net calcium absorption ($P = 0.66$) or net calcium retention ($P = 0.66$). ●, Black girls; ○, White girls.

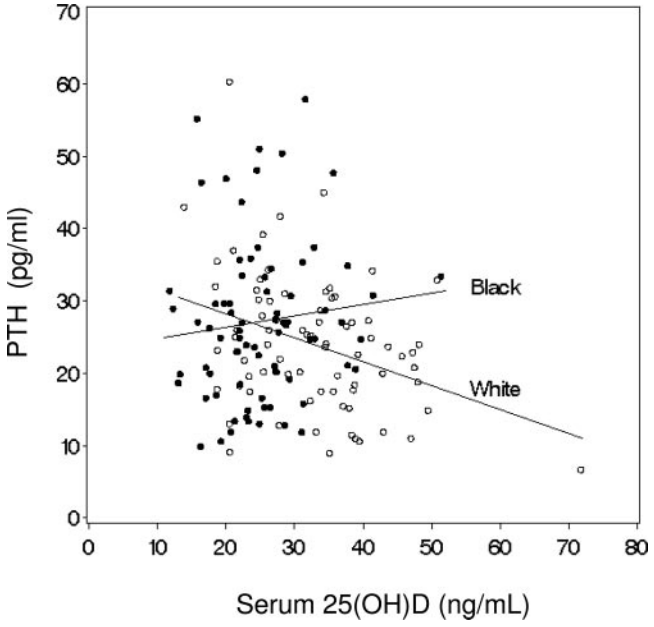

FIG. 3. The relationship of serum 25(OH)D and PTH varied with race ($P = 0.007$). The relationship was unchanged if the apparent outlier at high serum 25(OH)D with low PTH was omitted from the analysis. ●, Black girls; ○, White girls.

Calcium absorption was also not influenced by vitamin D status in White or in Black adolescent girls. This finding agrees with the findings in other studies in children that either show no relationship between calcium absorption and serum 25(OH)D (5, 21) or indeed a negative relationship (22). In 93 adolescents, there was no relationship between calcium absorption measured by stable double isotopes from a 300-mg calcium meal and serum 25(OH)D (5). In 16 healthy adolescents aged 9–16 yr old recruited in Beijing China during winter, serum 25(OH)D levels were low and showed a negative relationship with fractional calcium absorption measured by a double-isotope technique (22). However, these children also had very low calcium intakes, which were probably the explanation for the negative relationship. In adults, particularly the elderly, serum 25(OH)D is often found to be positively related to calcium absorption because both decrease with age (9, 23). Increasing serum 25(OH)D is associated with increasing absorption (24, 25) although only if there is a response in serum $1,25(OH)_2D$ (24).

Calcium supplementation reduces PTH, serum $1,25(OH)_2D$, and bone turnover in children (26). Our data supported a weak negative relationship between calcium intake and serum $1,25(OH)_2D$ ($r = 0.15$; $P = 0.079$; data not shown) but not PTH. Black girls had higher serum $1,25(OH)_2D$ levels than White girls and higher net calcium absorption. However, with calcium intake and race in prediction models, serum $1,25(OH)_2D$ did not contribute to the variation in calcium retention or net calcium absorption. A positive relationship between serum $1,25(OH)_2D$ and PTH and calcium fractional absorption in children has also been reported by Abrams *et al.* (5).

The inverse relationship between serum 25(OH)D and PTH reported in elderly adults (27, 28) and children (2–5) was observed only in White, but not Black, girls in our study. In the study of 307 Boston children aged 11–18 yr (4), there was no difference in the relationship between serum 25(OH)D and PTH between White (66% of subjects) and non-White adolescents (34% of subjects), but only 15% of the latter were Black (Abrams, S., personal communication). Only 13 of 93 children in the Houston cohort were Black (5). It is possible that if a wider range of serum 25(OH)D levels in Blacks were observed in our cohort, especially at higher levels, an inverse relationship would have been observed as for White girls. An inflection point in PTH concentration at approximately 32 ng/ml serum 25(OH)D has been observed in adults (27, 28) and in children (2). However, none of the studies in children conducted in the United States (4, 5), including ours, has found an inflection point, possibly because the French cohort included a larger number of vitamin

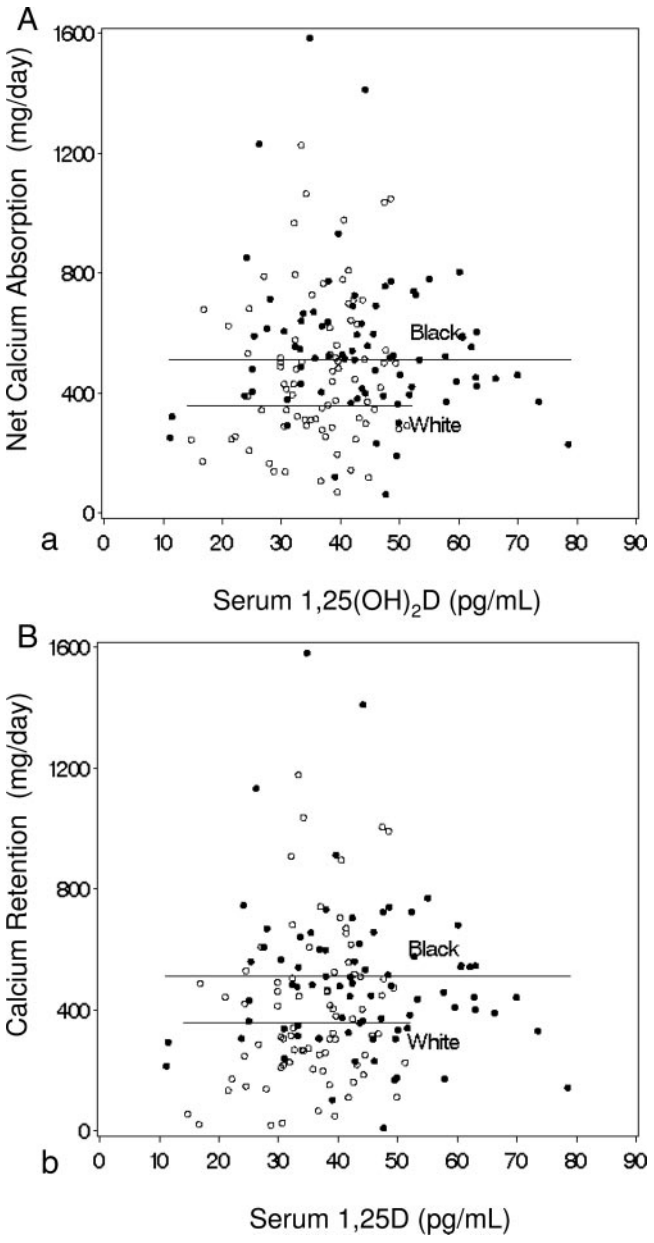

FIG. 4. Relationship of serum 1,25(OH)$_2$D and net calcium absorption (A) and net calcium retention (B) in adolescent girls. Black girls had higher net calcium absorption ($r^2 = 0.20$; $P = 0.0005$) and net calcium retention ($r^2 = 0.21$; $P = 0.0005$) than White girls. Serum 1,25(OH)$_2$D did not predict net calcium absorption or retention ($P = 0.67$). ●, Black girls; ○, White girls.

D-deficient children (2) than the U.S. studies. Indeed, others argue that in Whites, there is no plateau in the relationship between vitamin D status and PTH suppression (29). Whether PTH suppression is an advantage in children is uncertain. In a longitudinal study of 69 children aged 8–13 yr, bone mass and bone area accrual were highest in those with high-normal PTH and low-normal vitamin D status (30). In contrast, in our study, serum PTH was significantly negatively correlated with total body BMC ($r = -0.24$; $P < 0.05$) but only in White girls.

Despite significant differences in vitamin D status with race, there was no impact on measures of calcium utilization that would positively affect bone. This finding extends the previous literature because calcium intake was considered in the models.

The observed racial differences in BMC, calcium absorption and retention, and the relationship of PTH and vitamin D status may be explained by genetic effects, environmental differences, and by genetic-environment interactions. Genetic-environment interactions are apparent when individuals with different genetic profiles respond differently to a purely environmental factor such as diet (31). Genetic contributions from race are a small part of overall genetic variation. For example, the variation of serum 25(OH)D within race was much greater than the variation between Black and White girls (Fig. 1). Environment was controlled during the study period through controlled diet, physical activity, and sun exposure; however, these and other environmental factors before the study could not be controlled. Race-environmental interactions were found but were a relatively minor contributor to the variation in calcium retention. Race plus the one environmental variable, calcium intake, accounted for only 21% of the variation in calcium retention, leaving more than 75% of the variation unexplained, which perhaps is largely genetics. Racial differences in response to PTH have been studied in adults. Adult Black women are more resistant than White women to bone-resorbing effects of PTH (32) and the apparent sensitivity of BMD to vitamin D status (33). In adolescence, there is a tremendous biological drive for bone accretion which cannot be unraveled in adult studies. Although our data showed a different racial relationship between vitamin D status and PTH in adolescents, evaluating response to vitamin D will require an intervention study. Bone turnover is also lower in Black compared with White adults (34), but bone formation rates are higher in Black than White girls during peak growth rates (6). Biochemical markers of bone turnover are not sufficiently sensitive or specific to indicate these differences determined by calcium kinetic modeling and bone histomorphometry.

Our study has a number of limitations. It was cross-sectional, whereas longitudinal studies of vitamin D supplementation are needed to determine the dynamic response in calcium absorption and retention to increasing vitamin D status. The individual studies were performed over a 10-yr period, although we were able to show that the data were not affected by the time of study. It is conceivable that fasting PTH did not reflect integrated 24-h levels and that total PTH exposure may have been significantly higher in Black than White girls. Our study also has a number of strengths. The primary strengths are the relatively large numbers of Black and White pubertal girls studied; the controlled calcium intake; the wide range of vitamin D status, calcium intake, absorption and accretion; measurements during the summer season; and a narrow range of sexual maturity.

In summary, we have shown that calcium intake and race, but not vitamin D status, predicted calcium utilization. Black girls have higher calcium absorption and retention than White girls, despite lower vitamin D status. Vitamin D status does not predict calcium utilization in healthy Black or White adolescent girls at the time of their peak growth in the skeleton. Although serum threshold levels have been suggested that define vitamin D deficiency and above that prevent overt rickets (*i.e.* serum

TABLE 3. Predictors of calcium retention, urinary calcium, fecal calcium, calcium absorption, and total body BMC in adolescent girls

		Coefficients					
r^2	Racial Black-White difference	Ca intake (mg/d)	PMA (months)	25(OH)D (ng/ml)	1,25$(OH)_2$D (pg/ml)	NTx/Cr (nmol BCE/nmol Cr) or lean mass (g)[a]	Intercept
Predict calcium retention (mg/d)							
0.19[b]	153.30[b]	0.24[b]					67.74
0.21[b]	159.63[b]	0.22 [b]	NS				110.06
0.21[b]	167.08[b]	0.23[b]	NS	NS			73.6
0.21[b]	154.71[b]	0.23[b]	NS		NS		82.44
0.29[b]	196.53[b]	0.18[b]	NS			0.40[c]	−53.77
Predict urinary calcium (mg/d)							
0.13[b]	−32.92[b]	NS					72.70
0.18[b]	−34.87[b]	NS	1.10[c]				59.62
0.19[b]	−31.89[b]	NS	1.10[b]	NS			45.02
0.19[b]	−34.08[b]	NS	1.08[b]		NS		64.07
0.30[b]	−40.40[b]	0.028[c]	0.90[c]			−0.065[b]	77.91
Predict fecal calcium (mg/d)							
0.69[b]	−120.38[c]	0.75[b]					−140.43
0.69[b]	−124.76[c]	0.76[b]	NS				−169.68
0.69[b]	−135.18[c]	0.75[b]	NS	NS			−118.62
0.69[b]	−120.63[c]	0.75[b]	NS		NS		−146.51
0.71[b]	−156.13[b]	0.79[b]	NS			−0.33[c]	−24.14
Predict net Ca absorption (mg/d)							
0.19[b]	114.15[c]	0.24[b]					155.31
0.20[b]	118.53[c]	0.23[b]	NS				187.98
0.20[b]	126.60[c]	0.24[b]	NS	NS			148.64
0.20[b]	115.86[c]	0.23[b]	NS		NS		172.71
0.19[b]	118.15[c]	0.23[b]	NS			NS	193.13
Predict total body BMC (g)							
0.04[c]	NS	NS					2196
0.33[b]	NS	NS	22.05[b]				1938
0.33[b]	NS	NS	21.85[b]	NS			2087
0.33[b]	NS	NS	21.65[b]		NS		1985
0.84[b]	NS	NS	7.26[b]			0.07[b]	−616

For example, at menarche, the mean total BMC is 1878 g and increases by 22 g for every additional month PMA. BCE, Bone collagen equivalent; NS, not significant.

[a] Lean mass for total body BMC; NTx/Cr for all others.

[b] $P < 0.01$; n = 142–158.

[c] $P < 0.05$; n = 142–158.

25(OH)D < 11 ng/ml) (35), to date, there is no evidence related to calcium absorption or retention to support a cutoff for vitamin D insufficiency in children and adolescents.

Acknowledgments

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This work was funded by National Institutes of Health Grants AR40553 and HD36609.

Disclosure Summary: C.W. is on the Boards of Wyeth Global Nutrition and Pharmavite. Other authors have nothing to declare.

References

1. **Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C** 2000 Peak bone mass. Osteoporos Int 11:985–1009
2. **Guillemant J, Taupin P, Le HT, Taright N, Allemandou A, Pérès G, Guillemant S** 1999 Vitamin D status during puberty in French healthy male adolescents. Osteoporos Int 10:222–225
3. **El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Veith R** 2006 Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab 91:405–412
4. **Gordon C, DePeter KC, Feldman HA, Grave E, Emans SJ** 2004 Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med 158:531–537
5. **Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO** 2005 Relationships among vitamin D levels, parathyroid hormone and calcium absorption in young adolescents. J Clin Endocrinol Metab 90: 5576–5581
6. **Bryant RJ, Wastney ME, Martin BR, Wood O, McCabe GP, Morshidi M, Smith DL, Peacock M, and Weaver CM** 2003 Racial differences in bone turnover and calcium metabolism in adolescent females. J Clin Endocrinol Metab 88:1043–1047
7. **Hui SL, DiMeglio LA, Longcope C, Peacock M, McClintock R, Perkins AJ, Johnston Jr CC** 2003 Difference in bone mass between black and white American children: attributable to body build, sex hormone levels, or bone turnover? J Clin Endocrinol Metab 88:642–649
8. **Gilsanz V, Skaggs DL, Kovanlikaya A, Sayre J, Loro ML, Kaufman F, Korenman SG** 1998 Differential effect of race on the axial and appendicular skeletons of children. J Clin Endocrinol Metab 83:1420–1427

9. **Peacock M, Hordon L** 1989 Femoral fracture: the role of vitamin D. In: Kleerekoper M, Krane SM, eds. Clinical disorders of bone and mineral metabolism. New York and Basel, Switzerland: Mary Ann Liebert; 26–271
10. **Willis CM, Laing EM, Hall DB, Hausman DB, Lewis RD** 2007 A prospective analysis of plasma 25-hydroxyvitamin D concentration in white and black prepubertal females in the south-eastern United States. Am J Clin Nutr 85: 124–130
11. **Braun M, Palacios C, Wigertz K, Jackman LA, Bryant RJ, McCabe LD, Martin BR, McCabe GP, Peacock M, Weaver CM** 2007 Racial differences in skeletal calcium retention in adolescent girls on a range of controlled calcium intakes. Am J Clin Nutr 85:1657–1663
12. **Wastney ME, Zhao Y, Weaver CM** 2006 Kinetic studies. In: Calcium in human health. Weaver CM, Heaney RP, eds. Totowa, NJ: Humana Press; 83–93
13. **Jackman LA, Millane SS, Martin BR, Wood OB, McCabe GP, Peacock M, Weaver CM** 1997 Calcium retention in relation to calcium intake and postmenarcheal age in adolescent females. Am J Clin Nutr 66:327–333
14. **Palacios C, Wigertz K, Martin B, Jackman L, Pratt JH, Peacock M, McCabe G, Weaver CM** 2004 Sodium retention in Black and White female adolescents in response to salt intake. J Clin Endocrinol Metab 89:1858–1863
15. **Wigertz K, Palacios C, Jackman L, Martin BR, McCabe LD, McCabe GP, Peacock M, Pratt JH, Weaver CM** 2005 Racial differences in calcium retention in response to dietary salt in adolescent girls. Am J Clin Nutr 81:845–850
16. **Tanner J** 1962 Growth at adolescence. Oxford: Blackwell
17. **Allen LH, Reyonds WL, Margen S** 1979 Polyethylene glycol as a quantitative fecal marker in human nutrition experiments. Am J Clin Nutr 32:427–440
18. **Efron B, Tibshirani RJ** 1993 An introduction to the bootstrap. New York: Chapman, Hall
19. **Stein EM, Laing EM, Hall DB, Hausman DB, Kimlin MG, Johnson MA, Modlesky CM, Wilson AR, Lewis RD** 2006 Serum 25-hydroxyvitamin D concentrations in girls aged 4–8 y living in the southeastern United States. Am J Clin Nutr 83:75–81
20. **Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R** 2005 Estimates of optimal vitamin D status. Osteoporos Int 16:713–716
21. **Graff M, Thacher TD, Fischer PR, Stadler D, Pam SD, Pettifer JM, Isicher CO, Abrams SA** 2004 Calcium absorption in Nigerian children with rickets. Am J Clin Nutr 80:1415–1421
22. **Lee WT, Cheng JC, Jiang J, Hu P, Hu X, Roberts DC** 2002 Calcium absorption measured by stable calcium isotopes (^{42}Ca, ^{44}Ca) among northern Chinese adolescents with low vitamin D status. J Orthop Surg 10:61–66
23. **Heaney RP, Recker RR, Stegman MR, May AJ** 1989 Calcium absorption in women: relationship to calcium intake, estrogen status, and age. J Bone Miner Res 4:469–475
24. **Hordon L, Peacock M** 1987 Vitamin D metabolism in women with femoral neck fracture. Bone Miner 2:413–426
25. **Heaney RP, Dowell S, Hale CA, Bendich A** 2003 Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 22:142–146
26. **Slemenda CW, Peacock M, Hui S, Zhao L, Johnston Jr CC** 1997 Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. J Bone Miner Res 12:676–682
27. **Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PH** 1992 Vitamin D3 and calcium to prevent hip fractures in elderly women. N Engl J Med 327:1637–1642
28. **Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vanwakas E, Dick JM, Prince RL, Finkelstein JS** 1998 Hypovitaminosis D in medical inpatients. N Engl J Med 338:777–783
29. **Veith R, El-Hajj Fuleihan G** 2005 There is no lower threshold level for parathyroid hormone as 25-hydroxyvitamin D concentrations increase. J Endocrinol Invest 28:183–186
30. **Tylavsky FA, Ryder KM, Li R, Park V, Womack C, Norwood J, Carbone LD, Sheng S** 2007 Preliminary findings: 25(OH)D levels and PTH are indications of rapid bone accretion in pubertal children. J Am Coll Nutr 26:462–470
31. **Walker MD, Novotny Rachel, Bilezikian JP, Weaver CM** 2008 Race and diet interactions affect the acquisition, maintenance, and loss of bone. J Nutr 138: 1256S–1260S
32. **Cosman F, Morgan DC, Nieves JW, Shen V, Lucky M** 1997 Resistance to bone resorbing effects of PTH in black women. J Bone Miner Res 12:958–966
33. **Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B** 2004 Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 116:634–639
34. **Parisien M, Cosman F, Morgan D, Schnitzer M, Liang X, Nieves J, Forese L, Lucky M, Meier D, Shen V, Lindsay R, Dempster DW** 1997 Histomorphometric assessment of bone mass, structure, and remodeling: a comparison between health black and white premenopausal women. J Bone Miner Res 12:948–957
35. **Specker BL, Ho ML, Oesteich A, Yin TA, Shui QM, Chen XC, Tsang RC** 1992 Prospective study of vitamin D supplementation and rickets in China. J Pediatr 120:733–739