Racial differences in skeletal calcium retention in adolescent girls with varied controlled calcium intakes\textsuperscript{1–3}

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ABSTRACT
Background: Higher bone mass in blacks than in whites has been related to greater calcium utilization efficiency. Dietary calcium requirements for maximal skeletal calcium accretion during puberty may differ between the races.

Objective: This study compared the relation between calcium intake and calcium retention in black and white adolescent girls.

Design: A range of controlled calcium intakes (760–1981 mg Ca/d) were used in 3-wk controlled balance studies. Some subjects were studied more than once; a total of 182 observations from 55 black girls and 66 white girls were analyzed.

Results: Blacks had 185 ± 32 mg/d greater mean skeletal calcium retention than did whites (P < 0.0001) at all calcium intakes as a result of significantly greater net calcium absorption (P < 0.001) and lower calcium excretion (P < 0.0001).

Conclusions: Dietary calcium requirements did not differ with race. Higher calcium retention at all calcium intakes during adolescence may underlie the higher bone mineral content of adult blacks than of adult whites. \textit{Am J Clin Nutr} 2007;85:1657–63.

KEY WORDS Black persons, white persons, skeletal calcium, dietary calcium, adolescents

INTRODUCTION
Blacks have higher bone mineral density (BMD) than do whites \textsuperscript{1}, a difference that is present even during childhood \textsuperscript{2, 3}. Adolescence is a period of particular importance for skeletal growth because up to half of adult bone mass is accrued during this short period \textsuperscript{4}. Dietary calcium requirements of adolescents in the United States and Canada were set by the Institute of Medicine to optimize skeletal calcium retention on the basis of data from whites \textsuperscript{5}; however, the intake required to maximize bone mass may vary by race.

Our group has determined from metabolic balance studies the relation between dietary calcium intake and skeletal calcium retention in white girls \textsuperscript{6}. In a study on a calcium intake of 1100–1300 mg/d, we found that black girls had 72% greater skeletal calcium retention, 70% higher calcium absorption efficiency, and 46% lower urinary calcium excretion than did white girls \textsuperscript{7}. These results agree with those from other studies \textsuperscript{8, 9}.

The aims of the present study were to establish the relation between calcium retention over a wide range of calcium intakes in adolescent girls by collating data obtained from calcium balance studies conducted between 1990 and 2000 that used the same protocol and to compare the relations in white and black girls.

SUBJECTS AND METHODS

Subjects
Healthy adolescent girls were recruited as described previously \textsuperscript{6, 7, 10, 11}. Health was determined by medical history questionnaire, physical examination, and blood biochemistry. So that mean sexual maturity did not differ significantly between study groups within the study, black and white subjects were selected by utilizing questionnaires administered by a research coordinator to determine postmenarcheal age and Tanner stage \textsuperscript{12}. Race was established by the race of parents and grandparents as stated by subjects.

Total-body BMD and bone mineral content and total bone calcium were measured by using dual-energy X-ray absorptiometry [(DXA) Lunar Corp, Madison, WI]. Height and weight with the subject lightly clad and without shoes were recorded at the time of the DXA measurement. Subjects were studied on a controlled diet for 3-wk periods during the summer. Subjects lived in campus housing at Purdue University and participated in the usual activities associated with a camp environment.

Both the subjects and their guardians were fully informed about the study protocol. Subjects gave written assent, and guardians gave their written consent for participation. The studies were approved by the institutional review boards of Purdue University, Indiana University–Purdue University Indianapolis, and Clarian Health.

Dietary intake and analysis
Dietary intake was controlled by using a 4-d cycle menu, and subjects consumed only the foods prepared by staff. 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 by the administration of calcium-fortified juice and supplements to achieve intakes ranging from 760 to 1981 mg Ca/d. Beverage


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glasses containing calcium-rich fluids such as milk and juice were rinsed with deionized water, and the rinse was also consumed. Subjects were encouraged to completely consume all food. Food items not consumed within the designated mealtime were collected and weighed, and the intake was adjusted appropriately. The meal composite was prepared at the same time and to the same specifications as the meals; it was then frozen. Meal composites were thawed, homogenized, and freeze-dried for analysis (FTS Systems Inc, Stone Ridge, NY).

Sample collection and analysis
During each 3-wk balance study, each subject collected all of her urine and feces (6, 7, 10, 11). Urine was collected in acid-washed containers and pooled as 24-h collections, which ended with the rising collection the morning of each day. Fecal samples were collected in previously weighed containers and immediately frozen. Fecal samples were pooled as 24-h collections and diluted with concentrated hydrogen chloride and deionized water for homogenization by a stomacher (Tekmar, Cincinnati, OH). Aliquots were sampled in triplicate, dried at 48 °C, ashed at 600 °C, and diluted with 3% HNO3 for measurement of calcium. Diet samples were prepared similarly. Diet, fecal, and urinary samples were measured for calcium content by using atomic absorption spectroscopy according to National Bureau of Standards reference standards for monitoring quality control as described previously (6, 7, 10, 11).

Compliance measures
Urinary creatinine excretion was used both to assess urine collection compliance according to a constant daily urinary creatinine excretion and to adjust for 24-h collections. Urinary creatinine was measured on a Cobas Mira Plus (Roche Diagnostic Systems, Branchburg, NJ).

Fecal polyethylene glycol ([PEG] Dow Chemical Co, Midland, MI) excretion was used to assess fecal collection compliance. Each subject ingested 6 gelatin capsules (2 capsules with each meal). Capsules were assembled by hand, and each gelatin capsule contained 500 ± 5 mg PEG. PEG was analyzed by using a turbidimetric assay (13). All samples were analyzed in duplicate.

Skeletal calcium retention calculation
Calcium retention was calculated by subtracting fecal and urinary calcium excretion from dietary calcium intake. The first week of each 3-wk study was regarded as an equilibration period. A balance period began the day after the first fecal sample after the equilibrium period and ended on the last day with a fecal sample, and calcium retention was averaged over that period. Percentage net calcium absorption was calculated as intake minus fecal excretion divided by intake × 100.

Hormone and biomarkers of bone metabolism
Blood was drawn after an 8-h overnight fast during each 3-wk study period. Serum was analyzed for parathyroid hormone (PTH) 1–84, measured by a 2-site immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA; CV: 7.1%); insulin-like growth factor (IGF) was measured by using an enzyme-linked immunosorbent assay ([ELISA] Diagnostic Systems Laboratories, Webster, TX; CV: 7.9%); osteocalcin was measured by immunoradiometric assay (CV: 7.1%) (14); bone-specific alkaline phosphatase (BAP) was measured by ELISA (CV: 4.1%) (Quidel, San Diego, CA); 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D] by extraction and HPLC separation (CV: 11.4% and 10.1%, respectively).

Statistical analysis
Characteristics of black and white subjects were compared by using t tests assuming equal variances. When the variances of the 2 groups were markedly different, the results were verified by using a procedure that allows for different variances. In all instances, the results were the same. Because the relation between calcium intake and calcium retention differed at very high intakes, we excluded from our analysis 6 observations, all on whites, who had intakes >2000 mg/d. With this exclusion, the relation is approximately linear, and we used the SAS Mixed procedure as the basic analytic tool. The procedure accounts for correlation in multiple observations from the same subject. SAS procedures GLM and Mixed gave similar results; reported values are for GLM. The primary analysis examined racial differences in the relation between calcium intake and retention. Additional explanatory variables, including interactions, were examined within the context of this model. The same analytic framework was used to analyze fecal and urinary calcium excretion and net calcium absorption. SAS statistical software (version 9; SAS Institute, Cary, NC) was used for all analyses.

RESULTS
Several girls participated in ≥2 study periods, for a total of 84 observations in 55 black girls and 98 observations in 66 white girls. Physical characteristics, BMD, serum PTH, insulin-like growth factor, osteocalcin, BAP, and vitamin D metabolites are shown in Table 1. White girls and black girls were matched for sexual maturity within each study, and consequently, white girls were chronologically older than black girls (12.9 and 12.1 y, respectively; P < 0.001). Black girls had higher total BMD than did white girls (1.09 and 1.03, respectively; P < 0.001). Previous calcium intakes were greater in white than in black girls. There were no significant differences in any of the other variables.

The relation between calcium intake and calcium retention in blacks and whites was approximately linear, with blacks having significantly (P < 0.0001) higher retention than whites (Figure 1). The slopes did not differ significantly—it, there was no interaction between race and calcium intake (P = 0.86). In a model that assumes equal slopes, calculations were made with the use of the following equations:

\[
\text{Calcium retention (blacks) = 194.26 + 0.247 × calcium intake (mg/d)} \quad (1)
\]

and

\[
\text{Calcium retention (whites) = 9.75 + 0.247 × calcium intake (mg/d)} \quad (2)
\]

where P < 0.001 for the common slope. The difference in the intercepts, 184.51 ± 31.98 (P < 0.0001) represents the estimated average retention difference (in mg/d) across all calcium intakes within the range studied.
TABLE 1
Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Black subjects (n = 55)</th>
<th>White subjects (n = 66)</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>12.1 ± 1.1 (10.0–14.5)</td>
<td>12.9 ± 1.1 (11.0–15.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.9 ± 7.9 (141.0–173.4)</td>
<td>157.6 ± 6.6 (141.0–177.0)</td>
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<tr>
<td>Weight (kg)</td>
<td>54.7 ± 12.5 (32.0–84.3)</td>
<td>55.9 ± 16.4 (25.0–108.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 4.0 (15.6–30.9)</td>
<td>22.0 ± 5.7 (12.6–42.7)</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>3.8 ± 0.90 (1.0–5.0)</td>
<td>3.7 ± 0.91 (1.0–5.0)</td>
</tr>
<tr>
<td>Postmenarcheal age (mo)</td>
<td>10.1 ± 12.6 (0.0–44.0)</td>
<td>8.1 ± 14.1 (0–45.3)</td>
</tr>
<tr>
<td>Percentage lean body mass (%)</td>
<td>74.0 ± 9.7 (53.2–90.4)</td>
<td>70.7 ± 10.8 (44.9–94.4)</td>
</tr>
<tr>
<td>Total bone mineral density (g/cm³)</td>
<td>1.09 ± 0.10 (0.87–1.34)</td>
<td>1.04 ± 0.10 (0.81–1.30)</td>
</tr>
<tr>
<td>Total bone mineral content (g)</td>
<td>2232 ± 489 (1278–3479)</td>
<td>2089 ± 498 (1011–3675)</td>
</tr>
<tr>
<td>Total-body bone Ca (g)</td>
<td>845 ± 185 (485–1322)</td>
<td>793 ± 189 (384–1396)</td>
</tr>
<tr>
<td>1.25(OH)₂D (pg/mL)</td>
<td>39.5 ± 16.7 (11.2–78.6)</td>
<td>35.6 ± 7.6 (16.9–49.4)</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>31.9 ± 11.2 (13.1–63.1)</td>
<td>33.8 ± 10.6 (13.9–71.8)</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>27.9 ± 11.8 (9.9–57.9)</td>
<td>26.0 ± 10.7 (6.7–72.2)</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>38.5 ± 26.9 (5.6–133.3)</td>
<td>43.6 ± 27.7 (0.0–147.0)</td>
</tr>
<tr>
<td>BAP (ng/mL)</td>
<td>70.2 ± 33.3 (21.0–184.8)</td>
<td>87.7 ± 62.5 (3.8–323.0)</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>452.0 ± 130.7 (158.4–699.4)</td>
<td>396.6 ± 128.0 (188.0–610.5)</td>
</tr>
<tr>
<td>Previous calcium intakes (mg/d)¹⁰</td>
<td>654 ± 262 (204–1363)</td>
<td>925 ± 398 (248–1741)</td>
</tr>
</tbody>
</table>

¹ All values are ± SD; range in parentheses. 1,25(OH)₂D, 25-hydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; BAP, bone-specific alkaline phosphatase; IGF-1, insulin-like growth factor 1.
² P < 0.002 for the difference by t test.
³ For blacks and whites, respectively, n = ⁸⁵ and ⁶⁶, ⁵⁵ and ⁵⁶, ⁵₂ and ⁵¹, ⁵₂ and ⁵⁵, ⁵₂ and ⁶⁵, ⁷⁵ and ⁶², ⁶⁰ and ⁶², ⁹⁵ and ²⁷, and ²⁶⁹ and ³⁹.

With the use of a model with race and calcium intake as the major predictors of retention, the effects of additional explanatory variables were examined. From this analysis, postmenarcheal age (in mo) emerged as a significant predictor of retention (b = −3.92 ± 1.24 mg·d⁻¹·mo⁻¹; P < 0.002). Thus, each additional month after menarche was associated with a 3.92-mg/d decrease in calcium retention. This relation applied equally to blacks and whites and across the range of calcium intakes used. The correlation between postmenarcheal age and IGF-1 was 0.19 (P < 0.05), and serum IGF-1 was also negatively associated with retention (b = −0.424 ± 0.207; P < 0.05). Calcium intake explained 12.3% of the variation in calcium retention. Race explained an additional 13.7% of the variation, and postmenarcheal age explained an additional 3.9%. Alternatively, if serum IGF-1 was used in the model in place of postmenarcheal age, an additional 0.35% of the variability in calcium retention was explained. Prior calcium intake was not significant in the model.

The relation between urinary calcium intake and calcium excretion in both races was approximately linear: blacks had lower net calcium absorption than whites (Figure 3), although the slopes did not differ significantly (P = 0.17). In a model that assumes equal slopes, calculations were made with the use of the following equations:

Net calcium absorption (blacks) = 62.26

\[ \text{Net calcium absorption (blacks)} = 62.26 - 0.0128 \times \text{calcium intake (mg/d)} \]  

(5)

and

Net calcium absorption (whites) = 49.11

\[ \text{Net calcium absorption (whites)} = 49.11 - 0.0128 \times \text{calcium intake (mg/d)} \]  

(6)

where P < 0.001 for the common slope. The estimated average net calcium absorption difference was 13.15 ± 2.85% (P < 0.001). Neither postmenarcheal age nor IGF-1 was significant when added to this model. Calcium intake explained 9.4% of the variation in net calcium absorption, and race explained an additional 9.8%.

Calcium-regulating hormones, serum PTH, and 1,25(OH)₂D and 25(OH)D, were not significant predictors of calcium retention in either race. Nor were markers of bone turnover, osteocalcin, or BAP, which are significant predictors of racial differences in calcium retention.
DISCUSSION

The high daily skeletal calcium retention rates we found in these balance studies in adolescents reflect the well-established fact that ≈40% of the total calcium phosphate mineral of the adult peak bone mass is deposited in the years around puberty (15, 16). The higher rates of calcium retention we found in blacks than in whites are in accord with the consistently documented finding that African Americans have higher peak bone mass than do white Americans (1, 17, 18). Indeed, in these samples of adolescent girls, we found that black girls already had significantly higher total BMD than did white girls (Table 1), which is in agreement with the findings from some other studies (2, 3). The differences between rates of skeletal calcium retention in black and white adolescents are highly likely to be largely due to genetic differences. This possibility is perhaps not unexpected, because a large proportion of the variation in adult peak bone mass in both whites and blacks is highly heritable (19–21).

We previously showed that a major factor determining skeletal calcium retention in white adolescents is the amount of calcium in the diet (6) and that there were significant differences in calcium intake between blacks and whites (7). In this current study of pooled data from our balance studies over a wide range of dietary calcium intakes, we found that the relation between dietary calcium intake and skeletal calcium retention was linear. The slopes between races were parallel; black girls had consistently higher average calcium retention—185 mg/d. Dietary calcium intake and race accounted for 26% of the variation in skeletal calcium retention.

The calcium intake associated with maximal calcium retention was used by the Dietary Reference Intake panel to set calcium requirements for adolescents in the United States and Canada based on data in white girls (5, 6). The relation between calcium intake and calcium retention was evaluated by using a nonlinear model (6), which showed a plateau in calcium retention in white girls at intakes >1300 mg Ca/d. A plateau in calcium retention at very high dietary calcium intakes is likely due to fecal calcium soap formation and reduced calcium absorption (22). No plateau in calcium retention was apparent for black girls. This may be because intakes >2 g/d were not tested in the black girls.

Racial differences in calcium retention and BMD from our studies are apparent by puberty. The magnitude of the increase in BMD as a person matures from Tanner stage 1 to Tanner stage 5 is greater in blacks than in whites (23). Earlier onset of sexual...
maturity has an earlier onset in black girls than in white girls (24), and postmenarchal age predicted an additional 3.9% of the variation in calcium retention. The effect of calcium intake on calcium absorption, retention, and excretion and racial differences in these variables were not affected by the concentrations of 25(OH)D, 1,25(OH)2D, or PTH, which suggested that these adolescents were vitamin D sufficient.

Calcium absorption, calcium deposition in bone, and calcium retention all peak in girls just before the onset of menarche (6, 25, 26). At that time, the bone calcium deposition rate is ≈5 times that of adulthood. We observed that indexes of sexual maturity were key predictors of calcium retention. As sexual maturity progressed, calcium retention decreased, and the time after menarche was negatively related to calcium retention.

Across all intakes, lower urinary calcium excretion was observed in black girls than in white girls, which is similar to results in earlier studies (7, 8, 10, 27). In the study by Abrams et al (8), the racial difference was observed only in premenarcheal girls, in contrast to the present study, in which racial differences occurred even at later stages of sexual maturity. Net calcium absorption was significantly higher in black than in white girls across all intakes, which is consistent with other studies using single calcium intakes (7, 8, 11). Thus, for any given calcium intake within the range studied (760–2195 mg Ca/d), black girls absorb and retain more calcium and excrete less calcium than white girls. However, for each unit of increase in calcium intake, black and white girls absorb and retain the same amount of calcium. For each additional 1-mg calcium intake, an average of 25% will be retained, 2% will be excreted in the urine, and 73% will be excreted in the stools. Thus, daily dietary calcium requirements are the same for both races.

The present study has several strengths. There was a large number of subjects, who were tested under highly supervised, controlled conditions. Furthermore, all girls were from the same geographic region and were tested in the same season of the year, which probably reduced variation. However, the variations in calcium absorption and retention among subjects at each calcium intake are wide. It may be argued that the method of calcium balance has a large variation. However, the daily collection of 24-h urinary over a 14-d period has a small error, and yet the variation among these subjects in urinary calcium was just as large as the variations in calcium absorption and retention. The mechanisms underlying the variation are unknown. Perhaps, like the differences between blacks and whites in calcium absorption, differences in calcium retention and urinary excretion may also be due to genetic variation. A limitation of our study is the
absence of values at calcium intakes > 2 g/d in black girls, which is necessary to establish a threshold intake. Thus, it cannot be ruled out that a plateau intake occurs at a higher calcium intake in black than white girls. Higher peak bone mass in black women than in white women, despite lower calcium intakes, suggests that, if our calcium intake recommendations slightly underestimate intake for maximal retention in black girls, bone health is not likely to be compromised.

We concluded that, at all calcium intakes, black girls retain more skeletal calcium than do whites, which may explain the higher peak bone mass in black adults than in white adults. However, the response to changes in calcium intake is similar in blacks and whites, and the calcium requirements are the same in both races.

The authors’ responsibilities were as follows—CMW, MP, GPM, and BRM: the design of the study; BM, CP, KW, LAJ, RJB, BRM, MP, and CMW: the conduct of the study and data collection; MG, LDM, GPM, MP, and CMW: analysis and interpretation of the data; and MB, MP, LDM, GPM, and CMW: manuscript preparation. MB was a doctoral student in the laboratory of CMM during the development of this work. None of the authors had a personal or financial conflict of interest.

REFERENCES