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Dietary Calcium Requirements Do Not Differ between Mexican-American Boys and Girls^{1,2}

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Abstract

Mexican Americans are an understudied ethnic group for determinants of bone health, although the risk of age-related osteoporosis is high in this rapidly growing sector of the U.S. population. Thus, the objective of the present study was to establish the dietary calcium requirements for bone health in Mexican-American adolescents by measuring calcium retention calculated from balance in response to a range of dietary calcium intakes and to determine predictors of skeletal calcium retention. Adolescents aged 12-15 y were studied twice on paired calcium intakes ranging from 600 to 2300 mg/d using randomized-order, crossover 3-wk balance studies. Skeletal calcium retention was calculated as dietary calcium intake minus calcium excreted in feces and urine over the last 2 wk of balance. A linear model was developed to explain the variation on calcium retention. Boys (n = 20) were taller and had higher lean mass, usual dietary calcium intake, bone mineral content, and serum alkaline phosphatase compared with girls, whereas girls (n = 20) had higher Tanner scores and greater fat mass. Calcium retention increased with calcium intake (P < 0.0001) and did not differ by sex (P = 0.66). In boys and girls considered together, calcium intake explained 33% of the variation in calcium retention. Serum alkaline phosphatase explained an additional 11% of the variation in calcium retention. Other variables measured, including urine N-telopeptide of type I collagen/creatinine ratio, Tanner score, serum parathyroid hormone and 25-hydroxyvitamin D, weight, height, and body mass index, did not contribute to the variance in calcium retention. In adolescence, calcium retention in both Mexican-American boys and girls was higher than studied previously in adolescent nonHispanic white girls. This trial was registered at clinicaltrials.gov as NCT01277185. J. Nutr. doi: 10.3945/jn.113.188318.

Introduction

Mexican Americans are an increasing proportion of the U.S. population (1) and have a high risk of osteoporosis (2). However, they are an understudied group for the determinants of bone health. Because adolescence is a period of rapid skeletal growth in which >40% of adult peak bone mass is acquired (3) and peak bone mass is a strong predictor of bone fragility later in life (4), it is important to establish the calcium requirements in Mexican-American adolescents to optimize their peak bone mass (5).

We reported previously the relation between calcium intake and skeletal calcium retention in non-Hispanic white (6,7), black (8,9), and Chinese (10) adolescents. Results from these studies show that non-Hispanic white girls retained 473 mg/d Calcium at the intake for maximal retention, which was set at 1300 mg/d (6). American white boys retained dietary calcium more efficiently than girls by ~170 mg/d across a broad range of calcium intakes (7). Chinese-American boys also had higher calcium retention by \sim 180 mg/d than girls at high calcium intakes (10). Although similar comparisons are not available for non-Hispanic black boys and girls, non-Hispanic black girls retained more calcium across calcium intakes than non-Hispanic white girls by 185 mg/d (9). Similar studies are not available for Mexican Americans.

The aim of the present study was to measure skeletal calcium retention as a function of calcium intake and to determine predictors of calcium retention in Mexican-American adolescent boys and girls, using a similar design to our previous studies in adolescents of other racial/ethnic groups (6–10). These data are essential to establish the calcium requirements for optimal peak bone mass in Mexican Americans.

Participants and Methods

Participants. Forty-five Mexican Americans, 23 girls aged 12–14 y and 22 boys aged 13–15 y, were recruited (Fig. 1) from schools and clinics in Indiana and Illinois with help from the Indiana Clinical and Translational Science Institute. Families responding to flyers and postcards were sent a questionnaire to ascertain whether the adolescents fulfilled the inclusion and exclusion criteria. Applicants were excluded from the study if they were aged <11 or >15 y or had a history of postmenarcheal

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FIGURE 1 Study design, enrollment, and retention.

amenorrhea, pregnancy or abortion, eating disorders, oral contraceptive use, tobacco use, malabsorptive disorders, bone, liver, and kidney disease, or hormonal abnormalities that may affect calcium absorption. Both parents and grandparents had to be of Mexican descent to be eligible for the study. The health of participants was determined by questionnaires, baseline physical examination, and serum biochemistry panel. Pubertal development was evaluated by a pediatric endocrinologist using the Tanner scoring of external genitalia (testicular or breast enlargement) and pubic hair development (11); these 2 scores were averaged. Postmenarcheal age (PMA)⁷ was defined as months after onset of periods relative to the first day of the study. All participants were studied under protocols approved by the Institutional Review Boards of Purdue University and Indiana University School of Medicine. Participants and their guardians gave informed consent before the study began.

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Design. The design was a randomized crossover metabolic balance study in which each participant was assigned to 1 of 10 groups corresponding to 5 paired low-high calcium intakes to cover a range of 600-2300 mg/d and 2 possible orders. The assigned treatments were written on paper, sorted, and selected 1 at a time. The results were entered into a spreadsheet, and participants were assigned to groups in this order as they were enrolled. The study directors performed the randomization, enrolled the participants, and assigned the participants to groups on the first day of the study; those performing the measurements were unaware of the assignments.

⁷ Abbreviations used: IGF, insulin-like growth factor; NTx/Cr, N-telopeptide of type I collagen/creatinine ratio; PEG, polyethylene glycol; PMA, postmenarcheal age; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

Each metabolic period consisted of a 3-wk balance session, separated by a 1-wk washout period in which participants consumed their self-selected diets. Each participant was studied on 1 amount of calcium intake during the 3 wk of metabolic period 1 and on another calcium amount during the 3 wk of metabolic balance period 2 with random order of high and low. Additional calcium was accomplished through an appropriated amount of orange juice unfortified and fortified with calcium citrate malate to provide the amount of assigned calcium intake, as described previously (6). This form of calcium was used to be able to compare directly with other racial/ethnic groups studied previously by our group.

Participants were housed in a campus residence hall that was converted into a metabolic unit during the summer of 2010. The study was conducted as a summer camp, with educational and recreational components, simulating a free-living environment. All meals, snacks, and beverages were provided, and all urine and feces were collected during the study. Serum samples were collected at baseline (day 0) and at the end of each balance period (day 20).

Diet. Usual diet was estimated from 6 24-h dietary recalls collected at home before the study began. These were analyzed using the Nutrition Data System for Research (database version, 2011, Nutrition Coordinating Center, University of Minnesota).

During the balance periods, the diet was completely controlled. A 4-d cycle menu with 3 meals and 2 snacks was served. The basal diet provided 600 mg/d Calcium and was fixed for protein (87 ± 19 g/d), fat $(82 \pm 16 \text{ g/d})$, fiber $(22 \pm 5 \text{ g/d})$, sodium $(3.6 \pm 0.5 \text{ g/d})$, phosphorus (620 \pm 112 mg/d), and vitamin D (~200 IU/d). This amount of vitamin D intake was used to compare directly with other racial/ethnic groups studied previously by our group; in addition, this is the mean intake in U. S. adolescents (12). Each menu was designed at 5 different calorie amounts, ranging from 1750 to 3000 kcal/d, to meet individual energy needs by adjusting the volume of caloric beverages and foods that did not provide additional calcium and that had the same macronutrient proportions as the basal diet. Food and beverages were prepared with deionized water and weighed to the nearest one-tenth of a gram. Beverage glasses were rinsed with deionized water, and the rinse was also consumed. Participants drank deionized water ad libitum. Duplicate composites of each of the meals of the day were prepared daily to test for variation in food batches. These were homogenized, freeze dried, ashed at 600°C, and analyzed for calcium and other minerals in duplicate using inductively coupled plasma optical emission spectroscopy (Optima 4300DV; PerkinElmer Life and Analytical Sciences). The measured calcium content of each daily composite was used for the balance calculation.

Compliance. Meals were consumed in a controlled environment. Participants were strictly supervised at all times to ensure compliance and to avoid consumption of other foods, food exchange, or discarded food. At each meal or snack, participants were served individually coded food containers for their specified diet. Items not consumed during meals were offered at the next meal or snack, but if not consumed the same day, were stored for later analysis.

Collecting excreta was monitored. Urinary compliance was assessed by daily analysis of creatinine. Urinary calcium excretion was normalized for creatinine excretion to adjust to 24-h pools or for incomplete collections, using each participants mean creatinine excretion for the days used to calculate calcium retention. Fecal compliance was assessed by recovery of a non-absorbable fecal marker, polyethylene glycol (PEG; molecular weight, ~3400; Dow Chemical). Each participant ingested 3 g of PEG every day in 6 gelatin capsules (2 capsules of 0.5 g of PEG with each meal). For fecal collection compliance, we eliminated fecal collections when PEG recovery was <50%, which corresponded to <15% of the total fecal samples collected.

Measurements. Body weight was recorded daily in the morning while wearing nightclothes and no shoes using an electronic scale (Health O Meter). Height was measured once without shoes using a wall stadiometer. BMI was calculated as kilograms per square meters. Total body bone mineral density, bone mineral content, total body calucin, fat mass, and lean body mass were measured once during the equilibration

week by dual-energy X-ray absorptiometry (iDXA software version 4.3e; Lunar Corporation).

Urine and feces were collected in acid-washed containers on a daily basis during each metabolic period and pooled as 24-h samples. These were processed and analyzed as described previously (6). PEG was analyzed using a turbidimetric assay (13).

Serum total calcium, phosphate, total alkaline phosphatase, and creatinine and urinary creatinine, phosphate, and calcium were measured by routine chemistry. Serum 25-hydroxyvitamin D [25(OH)D] was analyzed by LC-MS/MS analysis (Agilent Rapid Res 1200 LC system; Novilytic) (14). Parathyroid hormone (PTH) 1–84 was analyzed by a 2-site immunoassay (CV \pm 7.1%) (Nichols Institute Diagnostics). Insulin-like growth factor (IGF) 1 and IGF binding protein-3 were analyzed by ELISA (Diagnostic Systems Laboratories). Crosslinked N-telopeptide was measured in fasting urine collected after an overnight fast at the end of each balance period by ELISA (Ostex).

Retention calculation. The first 7 d of each balance period served as equilibration to the basal diet, and the next 14 d were used in the balance calculations. Calcium retention was calculated by subtracting urinary and fecal values from dietary calcium. A 1-d lag was used when calculating intake minus fecal excretion to accommodate the 19-h transit time in the gut. A balance period began and ended on a day when a fecal sample was collected and included all days between the beginning and end. The sum of intake minus the sum of excretion was averaged over the time period of compliance. Calcium retention was calculated using the following equation:

calcium retention = dietary calcium intake - [urinary calcium (normalized) + fecal calcium]

In addition, mean net percentage calcium absorption was calculated as calcium intake minus calcium excretion in feces times 100.

Statistical analysis. With this crossover design using 40 participants, each of whom is measured on 2 calcium intake amounts, we predicted 80% power to detect a shift of 109 mg/d in calcium retention from our previous study in non-Hispanic whites using a 2-sided t comparison (in the context of a model using intake to predict retention) with a 5% type I error. To estimate the variables of the linear model relating intake to balance, all data were used together. The use of a crossover paired design increases the power for analysis.

Two-sample *t* tests were used to compare the means of boys and girls for physical characteristics at baseline and for the means of the bone biomarkers. The relations between fecal calcium excretion and calcium intake and between urine calcium excretion and calcium intake were approximated by linear functions in models that included sex and the interaction between sex and intake. A data-smoothing procedure was used to examine the relation between calcium intake and calcium retention (primary outcome) for girls and boys separately. This led to the identification of a linear model that provided a similar fit to the intake and retention data. Residuals from the linear model were used to examine the possible contribution of sex, pubertal maturity (Tanner stage), PMA (in girls), height, BMI, lean and fat body mass, bone measures, serum PTH, 25(OH)D, IGF, calcium, total alkaline phosphatase, and urinary excretion of the N-telopeptide of type I collagen/creatinine ratio (NTx/Cr), expressed as logs. Statistical inference was performed using a linear approximation to the relation. An SAS procedure (NLMIXED) was used to account for the within-participant and between-participant variations. The same approach was used to examine fecal calcium excretion and urinary calcium excretion. All statistical analyses were performed using SAS software (version 9.2; SAS Institute).

Results

A total of 23 girls and 22 boys were randomly assigned (Fig. 1). One boy and 3 girls dropped out of the study within the first 6 d. In addition, 1 boy had very poor collection compliance, as determined from urinary creatinine excretion and fecal PEG

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TABLE 1	Baseline physical characteris	stics of the Mexican-
American b	bys and girls in the study ¹	

Characteristics	Boys (<i>n</i> = 20)	Girls (<i>n</i> = 20)
Age, y	14.1 ± 0.9	13.6 ± 1.0
Height, <i>cm</i>	170 ± 6.9	$158 \pm 5.1^{*}$
Weight, kg	77.2 ± 24.7	68.0 ± 18.3
Tanner stage ²	2.7 ± 1.0	$3.8 \pm 0.7^{*}$
PMA age, <i>mo</i>	—	18.4 ± 12.5
BMI (kg/m ²) classification (percentile for age), $\%$		
Underweight (<5th percentile)	0	0
Normal BMI (5th to 85th percentile)	35	25
Overweight or obese (≥85th percentile)	65	75
Lean body mass, %	63.7 ± 9.2	$57.1 \pm 6.3^{*}$
Fat body mass, %	32.8 ± 9.8	$39.5 \pm 6.8^{*}$
Usual calcium intake, <i>mg/d</i>	911 ± 362	$649 \pm 192^{*}$
TBMC, g	2527 ± 471	$2189 \pm 277^{*}$
TBMD, g/cm ²	1.09 ± 0.11	1.09 ± 0.10

¹ Values are means \pm SDs or percentages. *Different from boys, P < 0.05. PMA, postmenarcheal age; TBMC, total bone mineral content; TBMD, total body bone mineral density.

² Determined from Tanner stage of external genitalia, testicular size, and breast and pubic hair development (11).

recovery, and was not included. Thus, 20 girls and 20 boys were included in the final analyses.

Physical characteristics of the participants were measured at baseline (Table 1). Boys were taller and had higher lean mass, usual calcium intake, and bone mineral content compared with girls, whereas girls had higher Tanner scores and greater fat mass (P < 0.05). Most girls were postmenarcheal (17 of 20). In addition, most adolescents were overweight or obese (65-75%). Table 2 shows the biochemical markers of the participants at baseline, after 3 wk on low calcium diets, and after 3 wk on high calcium diets. There were no significant differences in IGF-1 or 25(OH)D concentrations between boys and girls at any time point. Boys had significantly higher concentrations of serum alkaline phosphatase and creatinine and significantly higher concentrations of urinary NTx/Cr. In addition, boys had significantly higher IGF binding protein-3 only after 3 wk on the high calcium diet. Girls had significantly higher concentrations of serum calcium at any time point compared with boys.

Urinary calcium excretion increased with calcium intake at a greater rate for girls than for boys (*P*-interaction < 0.05) (Fig.

2*A*). Fecal calcium increased with calcium intake for both boys and girls (P < 0.0001), with no sex differences (Fig. 2*A*). Paired individual data from participants studied at low and high calcium amounts demonstrated a high intraparticipant correlation for calcium retention (r = 0.74, P < 0.0001) (Fig. 2*B*). Calcium retention increased with calcium intake (P < 0.0001) (Fig. 2*C*). There were no differences in calcium retention between boys and girls (estimated difference, 38 ± 55 mg/d; P = 0.49). The mean net percentage calcium absorption was 42% on the low calcium intake and 39% on the high calcium intake (P < 0.001).

Although the relation between calcium retention and calcium intake is nonlinear, the range of the data used in our analysis was sufficient only to support fitting a linear model. Calcium intake was significant (P < 0.0001) and explained 33% of the variation in calcium retention, but sex was not significant (P = 0.49). The equation for predicting calcium retention from calcium intake was as follows:

calcium retention (mg/d) = 60.3 + 0.282× calcium intake (mg/d)

When the other possible explanatory variables were added to the model individually, only total alkaline phosphatase and NTx/Cr were statistically significant (P < 0.003 and P < 0.03, respectively) and explained an additional 11% and 10% of the variation, respectively. When both total alkaline phosphatase and NTx/Cr were included in the model, only total alkaline phosphatase was statistically significant. Height was not a significant predictor. Therefore, our final model, which explained 44% of the variation in calcium retention, was as follows:

calcium retention (mg/d) = 839 + 0.279[calcium intake (mg/d)] + 176[log total alkaline phosphatase(U/L)]

In addition, we developed models using data from the 20 Mexican-American boys reported here and 31 non-Hispanic white boys reported previously (7) to examine the effect of race/ ethnicity in boys. The explanatory variables examined, in addition to calcium intake, were serum IGF, total alkaline phosphatase, urine NTx/Cr, and Tanner score. In this model, calcium intake was statistically significant (P < 0.0001) and explained 25% of the variation in calcium retention, but race/ ethnicity was not significant (P = 0.80). When the other variables

TABLE 2 Biochemical bone markers at baseline and at the end of each 3-wk metabolic period under the low and high calcium intakes in Mexican-American boys and girls¹

	Baseline		Low calcium intake (631–1275 mg/d)		High calcium intake (1118–2157 mg/d)	
Biomarkers	Boys (<i>n</i> = 20)	Girls (<i>n</i> = 20)	Boys (<i>n</i> = 20)	Girls (<i>n</i> = 20)	Boys (<i>n</i> = 20)	Girls (<i>n</i> = 20)
Total serum alkaline phosphatase, U/L	241 ± 81	142 ± 53*	219 ± 69	133 ± 38*	224 ± 72	140 ± 60*
Serum Ca, <i>ng/dL</i>	9.91 ± 0.27	9.92 ± 0.28*	9.57 ± 0.23	9.83 ± 0.27*	9.60 ± 0.24	9.85 ± 0.33*
Serum creatinine, ng/dL	0.92 ± 0.09	$0.78 \pm 0.08^{*}$	0.90 ± 0.09	$0.77 \pm 0.06^{*}$	0.90 ± 0.10	$0.78 \pm 0.07^*$
Serum PTH, <i>pg/mL</i>	25.1 ± 7.5	26.7 ± 9.2	28.5 ± 10.7	31.6 ± 8.6	25.1 ± 10.2	27.8 ± 7.4
Serum IGF-1, $\mu g/L$	298 ± 75	302 ± 45	269 ± 72	293 ± 36	259 ± 67	285 ± 41
Serum IGFBP3, $\mu g/mL$	4.14 ± 1.44	3.27 ± 0.61	3.88 ± 1.19	3.27 ± 0.63	3.95 ± 1.27	3.12 ± 0.47*
Serum 25(OH)D, $\mu g/L$	24.7 ± 3.8	22.1 ± 5.3	26.6 ± 2.8	23.4 ± 6.6	26.6 ± 5.3	26.2 ± 5.9
Urinary NTx/Cr, nmol BCE/mmol creatinine	$694~\pm~391$	$354 \pm 165^{*}$	647 ± 347	$392 \pm 194^{*}$	$637~\pm~330$	$378 \pm 198^{*}$

¹ Values are means \pm SDs, n = 20. *Different from boys, P < 0.05. BCE, bone collagen equivalent; Ca, calcium; IGF-1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor binding protein-3; NTx/Cr, N-telopeptide of type I collagen/creatinine ratio; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

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FIGURE 2 Urinary and fecal calcium excretion, calcium retention, and dietary calcium intake at the end of each 3-wk metabolic period under the low and high calcium intakes in Mexican-American boys and girls. The relation between urinary and fecal calcium excretion and dietary calcium intake (milligrams per day) (*A*). A sex × calcium intake interaction in urinary calcium was found (*P*-interaction < 0.05); the prediction equation for urinary calcium excretion in girls was 70.69 + 0.043 × calcium intake and in boys was 56.26 + 0.017 × calcium intake. For fecal calcium, there was no sex × calcium intake interaction or a sex effect (*P*-interaction > 0.05); calcium intake was significant (*P* < 0.0001), and the prediction equation for fecal calcium excretion is $-122.4 + 0.69 \times$ calcium intake. Calcium retention as a function of calcium intake (mean and 95% CI for regression lines) (*B*). There was no sex × calcium intake interaction or sex

were added to the model individually, only IGF-1 was statistically significant (P < 0.02) and explained an additional 7% of the variation. Our final model, which explained 34% of the variation in calcium retention, was as follows:

calcium retention
$$(mg/d) = 1027 + 0.31 [calcium intake (mg/d)]$$

+ 0.189[log IGF - 1 (ng/mL)]

Similarly, we also developed models in girls using data from the 20 Mexican-American girls reported here and 35 non-Hispanic white girls reported previously (6) to examine the effect of race/ ethnicity. Here we examined PMA as an additional explanatory variable. In this model, calcium intake was statistically significant (P < 0.0001) and explained 15% of the variation in calcium retention. Race/ethnicity was also significant (P = 0.015), with Mexican-Americans girls retaining more calcium than non-Hispanic white girls across the range of intakes. This model explained 23% of the variation in calcium retention. PMA was negatively related and statistically significant when added to the model (P = 0.0006) and explained an additional 13% of the variation. Our final model, which explained 36% of the variation in calcium retention, was as follows:

calcium retention (mg/d) = 183 + 0.241[calcium intake (mg/d)]+172 [race] -5.21[PMA(months)]

Discussion

To our knowledge, this is the first study to determine the influence of a wide range of calcium intakes on calcium retention in Mexican-American adolescent boys and girls. Short-term balance studies (2-3 wk) measure changes in bone calcium because of the rapid adaptation in balance to changes in calcium intake and because 99% of the calcium in the body is in the skeleton. Thus, balance studies measured over a wide range of dietary calcium intakes can be used to establish calcium requirements during adolescence. In the present study, we found no sex differences in calcium retention in Mexican-American adolescents. In contrast, we found previously that calcium retention was higher in non-Hispanic white boys compared with white girls (7) and in Chinese-American boys compared with Chinese-American girls at high calcium intakes (10). This lack of racial difference in Mexican-American adolescents could be related to the substantially lower Tanner score in the Mexican-American boys compared with the girls. To test this, we ran regression models including several important predictors of bone accretion, but neither sex nor Tanner score were significant in the models.

Calcium intake explained 33% of the variation in calcium retention in Mexican-American boys and girls. This is higher compared with results in other groups tested in our laboratory using the same research protocols. In non-Hispanic white adolescents, calcium intake explained 15% of the variation in calcium retention in girls (6) and 22% in boys (15), and in Chinese-American girls, calcium intake explained 29% of the

effect in calcium retention (*P*-interaction > 0.05). Calcium intake was significant (P < 0.0001), and the prediction equation for calcium retention was 60.3 + 0.282 × calcium intake (milligrams per day). The 95% Cl for the sex difference was (-71, 147). Individual calcium retention on low and high calcium diets (milligrams per day) (*C*). Intraparticipant correlation was 0.76 (P < 0.0001).

variation in calcium retention; however, it is lower compared with Chinese-American boys, which was 53% (10). The other strong predictor of calcium retention in the Mexican Americans was total alkaline phosphatase, explaining an additional 11% of the variation. Total alkaline phosphatase is a marker of bone formation (16). This marker was not found to be a predictor of calcium retention in the other ethnic groups studied. Total alkaline phosphatase, unlike in the other ethnic groups studied, is a marker of bone formation. In non-Hispanic white girls, PMA was the other main predictor, explaining an additional 10% (negative relation) of the variation in calcium retention (6). In Chinese-American girls, estradiol explained an additional 15% (negative relation) of the variation in calcium retention (10). Therefore, it appears that, in non-Hispanic white and Chinese-American girls, calcium retention is negatively influenced by hormonal-related predictors but not in the Mexican-American girls. In non-Hispanic white boys, the other strong predictor was IGF-1, explaining an additional 12% of the variance (15), whereas in Chinese-American boys, testosterone explained 13% (10). Thus, there appears to be different factors in the samples studied by our group; alternatively, all these predictors may be reflecting pubertal growth, and, in different studies, 1 indicator may happen to be stronger than another.

To address whether there are race/ethnic differences in calcium retention in adolescents, we created models combining the previously reported data from non-Hispanic white boys and girls (6,7) with data from the Mexican-American boys and girls reported here. In boys, calcium intake explained 25% of the variation in calcium retention, but race/ethnicity did not explain this variance. Only IGF-1 was a significant predictor of the variation in calcium retention, explaining an additional 7% of the variation. IGF-1 is an important regulator of longitudinal bone growth during this period, because it stimulates proliferation and differentiation of chondrocytes at the epiphyses (17). IGF-1 peaks during puberty, closely matching peak bone accretion rates (18). Thus, this analysis showed that Mexican-American and non-Hispanic white boys have similar physiologic responses to calcium intake. In contrast, the model developed in girls showed that calcium intake is a weaker predictor of the variation in calcium retention compared with boys. Race was a significant predictor, which explained an additional 8% and PMA explained an additional 13% of the variation. Thus, Mexican-Americans girls retain more calcium than non-Hispanic white girls across the range of intakes by 172 \pm 45 mg/d, essentially the same amount of benefit of non-Hispanic white boys over white girls on the same calcium intakes $(171 \pm 38 \text{ mg/d})$ (9). In addition, as reported previously in other race/ethnic groups, bone biomarkers, vitamin D, PTH, and body size (weight, height, and BMI) were not significant predictors of calcium retention in these models in adolescents. In general, these models only explained 34–36% of the variance in calcium retention; therefore, it is likely that other factors must be involved.

Puberty is a period with high variability in calcium accretion. Each person has his or her own timing and height of peak bone accretion rate. Some participants were likely tested near their peak values, which is a transient period, whereas others were tested farther from their peak. As determined by Bailey et al. (19) in non-Hispanic white adolescents, the mean age of peak calcium accretion is 14.0 y in boys (12.0–15.9 y) and 12.5 y in girls (10.5–14.6 y). In addition, they estimated that 26% of adult calcium is laid down during the 2 y in adolescence, surrounding their peak skeletal growth. Similar data in Hispanics are not available; therefore, it is not known whether

Mexican-American boys and girls have similar ages of peak skeletal retention as whites or whether they have the same optimal bone accretion rates.

In this study, we also found that the mean net percentage of calcium absorption was significantly higher while on low calcium diets (42%) compared with high calcium diets (39%), as expected. It must be remembered that absorption varies with calcium intake. This will be confirmed from kinetic analysis for fractional calcium absorption.

This study has several strengths. Participants were studied under highly supervised, controlled conditions. Adolescents were from 1 geographic region and were tested in the same season of the year to directly compare sex differences. We tested different amounts of dietary calcium during the period of peak bone mass accrual, and balance was calculated daily from 24-h urine and fecal collections. A limitation was the short-term nature of this study, which precluded our ability to evaluate the effects of dietary calcium on actual changes in bone mass. IGF-1 was not assessed in non-Hispanic white girls. The results are limited to this narrow age and Tanner stage group. In addition, this is a relatively small sample of Mexican Americans, who were on average more overweight than the national average (20), which limits the generalizability to other Hispanic populations.

In conclusion, there were no differences in calcium retention between Mexican-American boys and girls, and the main predictors of calcium retention were calcium intake and total alkaline phosphatase. We reported previously sex differences in calcium retention in other race/ethnic groups and that growth regulators, rather than markers of bone turnover, were secondary predictors of calcium retention to calcium intake in these groups. When these data were combined with results in non-Hispanic white adolescents, we found that calcium retention was not different in boys, but Mexican-American girls have higher calcium retention compared with non-Hispanic white girls. The results presented here have the potential to help define racial/ethnic-specific calcium recommendations for behavior changes to maximize peak bone mass during adolescence and reduce the risk of osteoporosis later in life.

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