Calcium retention in relation to calcium intake and postmenarcheal age in adolescent females

Lisa A Jackman, Stephanie S Millane, Berdine R Martin, Olivia B Wood, George P McCabe, Munro Peacock, and Connie M Weaver

ABSTRACT  Achievement of maximal calcium retention during adolescence may influence the magnitude of peak bone mass and subsequently lower the risk of osteoporosis. Calcium retention is generally considered to reach a plateau at a certain calcium intake. To test this hypothesis, calcium balance was measured in 35 females with a mean (± SD) age of 12.7 ± 1.2 y (range: 12–15 y) who consumed 841 ± 153 to 2173 ± 149 mg Ca/d. Subjects ate a basal diet that included a fortified beverage containing different amounts of calcium citrate malate. Twenty-one subjects were studied at two dietary calcium intakes with use of a crossover design. Results from a previous study in 14 subjects who were studied at only one calcium intake were included in the data analysis. Calcium retention was modeled as a nonlinear function of calcium intake that included a parameter representing mean maximal retention. Mean maximal calcium retention was 473 mg/d (95% CI: 245, 701 mg Ca/d). At higher postmenarcheal ages, maximal calcium retention was lower but the intake required to achieve this was not affected. Calcium intake explained 79% and 6%, respectively, of the variation in fecal and urinary calcium excretion. Intake of 1200 mg Ca/d, the recommended dietary allowance for calcium published in 1989, resulted in a mean calcium retention that was 57% of the maximal value (95% CI: 25%, 89%). Intake of 1300 mg Ca/d was the smallest intake that allowed some adolescent females to achieve 100% of maximal calcium retention (95% CI: 26%, 100%). These data support the idea that calcium retention plateaus at a certain calcium intake although it continues to increase at intakes > 2 g/d. Am J Clin Nutr 1997;66:327–33.

KEY WORDS  Calcium intake, calcium retention, maximal calcium retention, calcium balance, requirements, calcium citrate malate, adolescent females, postmenarcheal age

INTRODUCTION  An important strategy in the prevention of osteoporosis is maximization of peak bone mass. It has been estimated that as much as 51% of peak bone mass is accumulated during pubertal growth in females (1). In an earlier study we applied a nonlinear model to assess cross-sectional total-body bone mineral content (TBBMC) in 246 young women (11–31 y) and reported that 95% of TBBMC was attained by 20 ± 2 y (± SEM) of age (2). Others who measured TBBMC cross-sectionally in 265 premenopausal white females aged 8–50 y found that TBBMC increased by only ≈4% from 18 to 50 y of age (3). Furthermore, in a longitudinal study (≤ 5 y) of 156 women aged 18.5–26 y at study entry, TBBMC increased by an average of only 1.2% y during the third decade of life and the rate of gain slowed with age (4).

Dietary calcium is an important factor in optimizing peak bone mass. Randomized controlled trials of calcium supplementation in children and adolescents found that increasing calcium intake increased bone calcium accretion during childhood and adolescence (5–8).

Matkovic and Heaney (9) suggested that calcium retention in adolescents reaches a plateau (threshold intake) at 1500 mg Ca/d. They used a two-component linear-regression model to assess data compiled from 133 calcium-balance studies in children aged 9–17 y that had been published since 1922 and calculated mean (± SEM) maximal calcium retention to be 396 ± 164 mg/d. However, the effect of chronologic and postmenarcheal age on calcium retention was not examined. Furthermore, the SEM for a calcium intake of 1500 mg/d was not stated and it is unclear whether the wide variability in retention values represented a true variation or resulted from pooling data from a variety of study designs and methods over wide intervals of time and age ranges of subjects.

The calcium intake of 1500 mg/d that resulted in maximal retention in the study by Matkovic and Heaney (9) exceeds the 1989 recommended dietary allowance (RDA) of 1200 mg/d for this age group (10), and this finding was instrumental in the 1994 decision by the National Institutes of Health (NIH) Consensus Conference Panel on Optimal Calcium Intakes to advise that the calcium intake value for adolescents be increased to 1200–1500 mg/d (11). Therefore, the purpose of this study was to describe the relation between calcium retention and dietary calcium intake in adolescent girls aged 12–15 y who had calcium intakes ranging from 841 to 2173 mg/d and were studied at one center.
SUBJECTS AND METHODS

Subjects

Adolescent girls were recruited from local schools and a screening questionnaire was completed by all applicants. Criteria for participation included the following: white female aged \( \geq 12 \pm 14 \) y; body mass index (BMI), \( \geq 15 \pm 85 \) percentile for age; no medical problems or use of medications, including oral contraceptives, that interfere with calcium metabolism; a usual calcium intake \( \geq 800 \) mg/d assessed by three 24-h dietary records; no history of pregnancy or abortion; no past or current eating disorder; and no history or current use of tobacco. Pubertal development was self-evaluated with use of the Tanner scale for sexual maturation (12). Height was measured with a wall-mounted stadiometer with subjects wearing light clothing and no shoes. Weight was measured with a calibrated electronic scale. Twenty-eight subjects were originally enrolled in the study.

Study protocols were approved by Use of Human Subjects Research Committees of Purdue University and Indiana University School of Medicine.

Study design and calcium-balance protocol

Subjects stayed in a sorority house on the Purdue University campus and participated in a planned program of educational and recreational activities. Two 21-d calcium-balance studies were conducted, with a 4-wk washout period between studies. The study had a crossover design: calcium balances were studied twice in the same subjects at one of four pairs of high and low calcium intakes in random order as shown in Table 1, which also shows the dietary calcium intake assigned to adolescent females studied previously at only one intake (13). Dietary calcium intakes above and below the 1200–1500 mg/d advised by the NIH Consensus Conference were selected. Subjects were stratified to groups on the basis of baseline serum osteocalcin concentration, BMI, and postmenarcheal age, all of which are known to influence calcium retention (14), and were blinded to their calcium intake.

The first 7 d of each balance period served as a period of adjustment to the basal diet. The last 14 d were the experimental periods. Calcium retention was determined as total intake minus total excreta through the last stool collection. Three grams of polyethylene glycol (PEG) (E3350; Dow Chemical Co, Midland, MI) was administered daily with meals (two capsules per meal) to all subjects to assess compliance and determine when steady state had been achieved. Each capsule contained 500 \( \pm 5 \) mg PEG (\( \bar{x} \pm SD \)) (15), a nonabsorbable fecal marker that is excreted in the liquid phase of feces.

Diet

Three meals and two snacks providing \( \approx 8368 \) kJ were served to subjects daily. A 6-d cycle menu of foods that appeal to adolescents was used. Subjects were required to consume all foods in the basal diet and to rinse beverage cups with deionized water. Discretionary dietary salt was not allowed. Subjects were allowed to consume deionized water, calcium-free lemonade that was reconstituted with deionized water, and flavored frozen-water treats (Popsicles; Good Humor-Breyers Ice Cream, Green Bay, WI) freely. Body weight was recorded daily.

Foods and beverages were prepared with deionized water and weighed to the nearest 0.1 g on digital scales. Duplicate composites of each day’s diet were prepared and stored at 0 °C for analysis of calcium, protein, fat, phosphorus, and magnesium. The average (\( \pm SD \)) calcium content of the basal diet for both balance periods was 799 \( \pm 163 \) mg/d. Dairy products provided most of the calcium in the basal diet.

Eight dietary calcium intakes ranging from 841 to 2173 mg/d were used. These intakes were achieved by serving each subject the basal diet plus a fruit-flavored beverage containing different amounts of calcium citrate malate (Procter and Gamble, Cincinnati). The beverage was weighed to the nearest 0.1 g into acid-washed color-coded plastic cups with lids. Approximately 840 mL beverage was consumed daily.

Analytic procedures

Acid-washed containers were used to collect urine in 24-h pools, beginning with the second voiding of the day and ending with the first voiding of the following day. Total urine volumes were measured daily and aliquots were acidified to a fixed concentration of 1% (by vol) with HCl and frozen at \(-10 ^{\circ} C\) for future analysis of calcium.

Individual fecal samples were collected, processed, and analyzed separately. Each sample was homogenized in a stomacher (Tekmar Co, Cincinnati) with deionized water in an amount equal to twice the weight of the fecal sample and concentrated HCl equal to 1% of the volume of water. Aliquots were frozen at \(-10 ^{\circ} C\) for future analysis of calcium and PEG. A turbidimetric assay was used to measure PEG in fecal homogenerate (16, 17).

Calcium in the diet and urine and fecal samples was measured by atomic-absorption spectrophotometry (5100 PC; Perkin-Elmer, Norwalk, CT). Freeze-dried dietary and fecal samples were reduced to ash in a muffle furnace at 600 °C for 72–96 h and diluted with 0.5 mol HCl/L containing 0.5% lanthanum as lanthanum chloride. Acidified urine samples were also diluted with lanthanum chloride solution. Durum wheat flour (reference material 8436; National Institute of Standards and Technology, Gaithersburg, MD) was assayed to ensure it contained 285 \( \pm 7 \) ppm Ca (\( \bar{x} \pm SD \)) (CV: 2.29%) compared with the certified value of 278 \( \pm 26 \) ppm after adjustment for moisture content.

Dietary composites were analyzed for phosphorus, nitrogen, and fat content. The colorimetric method of Murphy and Riley

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Dietary calcium intake in groups of female adolescents</td>
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<tr>
<td>Low dietary calcium</td>
</tr>
<tr>
<td>mg/d</td>
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<tr>
<td>Group</td>
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<tr>
<td>A ( (n = 10)^{2} )</td>
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<td>B ( (n = 3)^{2} )</td>
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<td>C ( (n = 3)^{2} )</td>
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<td>D ( (n = 5)^{2} )</td>
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<tr>
<td>E ( (n = 14)^{1} )</td>
</tr>
</tbody>
</table>

1 \( \bar{x} \pm SD \). Calcium determined by analysis.
2 Crossover design, randomized order.
3 Studied at only one calcium intake; reported previously by Weaver et al (13).
CALCIUM RETENTION IN ADOLESCENTS

(18) was used to measure dietary phosphorus concentration. The modified version of the Kjeldahl nitrogen determination method (19) was used to measure nitrogen content of the diets. Total dietary protein was estimated by multiplying grams of nitrogen by a conversion factor of 6.25 g protein/g N. Total daily fat was analyzed with use of the Association of Official Analytical Chemists Soxhlet extraction method (19). Each daily dietary composite was analyzed for all nutrients in triplicate. The diets were designed to be constant in other nutrients that may affect calcium retention. Analysis showed that the diets contained 1070 ± 342 mg P, 44 ± 11 g fat, and 61 ± 9 g protein (x ± SD). There were no significant differences between the balance periods in the analyzed values of these nutrients.

Statistics

A nonlinear-regression model was used to describe the relation between calcium intake and calcium retention. Many models were evaluated but the model that gave the best fit was as follows:

\[ Y = a e^{b L} (1 + e^{c}) \]  \hspace{1cm} (1)

where \( Y \) is retention and \( L \) is a linear function of intake, i.e., \( L = b + c \cdot \text{(intake)} \). The parameters in the model are \( a \), \( b \), and \( c \): the errors are assumed to be normally distributed with constant variance. The parameter \( a \) is the limiting value of retention for arbitrarily large intakes and represents the mean maximal calcium retention and the expression \( e^{b L} / (1 + e^{c}) \) represents the proportion of maximal retention for any given value of intake. The parameters \( b \) and \( c \) are the intercept and slope, respectively, of the linear function. Observations in the same individual at different intakes were assumed to be correlated and were transformed to uncorrelated observations by using a procedure similar to principal components analysis. The theory underlying these methods was described by Gallant (20), Seber and Wild (21), and McCabe (unpublished observations, 1996).

SEMs for the parameter estimators and functions of these estimators, such as retention or percentage of maximal retention for a given intake, were calculated by using standard methods. Plots of the residuals compared with postmenarcheal age suggested a linear relation with all postmenarcheal ages < 0 mo recorded as zero. Therefore, postmenarcheal age was included in the model in this way. Including postmenarcheal age as an additional linear term in the expression for \( L \) given above and other alternatives were also examined. A similar approach, which accounted for correlated errors, was used to model the linear relation between calcium intake and fecal and urinary calcium excretion. Plots, analysis of residuals, and regression diagnostics were used to develop these models. A \( t \) test was used to examine ratios of fecal calcium to fecal PEG. All calculations were performed with the SAS software package (SAS Institute, Inc. Cary, NC).

Previously reported data (13) from 14 adolescent girls consuming 1330 mg Ca/d in the same research protocol during the same season were included in the analyses along with the data from 21 subjects in the crossover study. Data from 7 of the 28 subjects originally enrolled in the crossover study were excluded from statistical analyses for both calcium intakes because of incomplete excreta collection or the subject's homesickness. Two additional subjects completed only the low-calcium portion of the study.

Data from subjects who were studied at two different intakes were examined for effect of the sequence of intake on retention (i.e., carryover effect). Because the high and low calcium intakes were not the same for all subjects, residuals from the nonlinear model were used for these calculations. The estimated mean (± SEM) change in calcium retention at the high intake due to having a low intake first was \(-24.3 \pm 100.2 \text{ mg/d} (95\% \text{ Cl: } -235.7, 187.1 \text{ mg/d})\). The estimated change in calcium retention at the low intake due to having a high intake first was \(-2.8 \pm 81.0 \text{ mg/d} (95\% \text{ Cl: } -173.6, 168.0 \text{ mg/d})\). There was no evidence of a carryover effect in these data. With 90% power, an effect of \(-150 \text{ mg Ca/d} \) on calcium balance would be detectable with this design.

To further examine for carryover effect, the model was rerun with all data from the second study deleted. The model parameters for optimal intake and maximal retention were very similar to those in the full model although, as expected, the SEMs were much larger. Therefore, we concluded that if there was an effect of sequence of calcium intake, it was not large enough to alter our analysis.

Paired \( t \) tests were used to compare differences between paired observations. Pearson correlations were used to determine relations between variables.

RESULTS

Mean (± SD) age, postmenarcheal age, sexual maturity stage, height, and weight of the subjects are shown in Table 2. The mean age was 12.7 ± 1.2 y.

A 7-d adjustment period was adequate to achieve equilibrium even at high calcium intakes because no significant differences in fecal calcium–fecal PEG ratios were observed between weeks 2 and 3 in either balance period. In one subject in group A consuming the high-calcium diet, equilibration did not occur until week 2; therefore, only the last week of the balance period was used in the analysis for this subject. In adolescents, equilibration for PEG (16) and for fecal calcium–fecal PEG ratio (13) usually occur after 1 wk. Fecal PEG recovery was 90 ± 10%.

Relations between urinary and fecal calcium excretion and calcium intake are shown in Figure 1. Although there was some nonlinearity in the relations, particularly for fecal calcium excretion at high intakes, linear regression accounted for

<table>
<thead>
<tr>
<th>Character</th>
<th>Value (± SD)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>12.7 ± 1.2</td>
</tr>
<tr>
<td>Postmenarcheal age (mo)</td>
<td>10.7 ± 11.9</td>
</tr>
<tr>
<td>Tanner sexual maturity stage (1-5)</td>
<td>3.8 ± 1.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>153.3 ± 23.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.5 ± 10.2</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.0 ± 3.6</td>
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\* x ± SD; n = 35 total; 21 subjects were in a crossover design and 14 were studied previously at only one calcium intake (13).
most of the variation in these excretion variables that could be explained by intake. The fitted equations were as follows:

\[
\text{Urinary excretion} = 57.5 + 0.025 \text{ intake} \quad (2)
\]

\[
(P < 0.001, \text{SEM of slope} = 0.003, r^2 = 0.06) \text{ and}
\]

\[
\text{Fecal excretion} = -56.3 + 0.75 \text{ intake} \quad (3)
\]

\[
(P < 0.001, \text{SEM of slope} = 0.04, r^2 = 0.79). \text{ The curvature in the relation between intake and excretion accounted for the leveling off of the intake-retention relation at high calcium intakes. The intrasubject correlations for urinary and fecal excretion were 0.57 and 0.97, respectively.}
\]

The relation between calcium intake and calcium balance was described by the nonlinear regression model shown in Figure 2 and the following formula:

\[
\text{Retention (mg/d)} = \frac{473 e^{2.77 \times 0.0025 \text{ intake}}}{(1 + e^{2.77 \times 0.0025 \text{ intake}})} \quad (4)
\]

The coefficient of intake in this model was significantly different from zero (one-sided \( P < 0.05 \)). The mean (± SEM) maximal calcium retention was 473 mg/d (95% CI: 245, 701 mg/d).

Percentages of predicted mean maximal calcium retention for calcium intakes ranging from 800 to 2100 mg/d are shown in Table 3. Calcium retention at each calcium intake was divided by mean maximal retention and 95% CIs are shown for each intake. This approach is useful for evaluating different calcium intakes.

Postmenarcheal age influenced the relation between calcium intake and calcium retention (Figure 4). This relation was described by the following formula:

\[
\text{Retention (mg/d)} = \frac{565.5(e^{2.11 + 0.0020 \text{ intake}})}{(1 + e^{2.11 + 0.0020 \text{ intake}})} + 18.2 - 7.42 \text{ PMA} \quad (5)
\]

where PMA refers to postmenarcheal age in months. Higher postmenarcheal age resulted in lower calcium retention at a given calcium intake and lower maximal calcium retention.

Mean (± SEM) maximal calcium retention was 584 ± 165 mg/d, 510 ± 161 mg/d, and 435 ± 160 mg/d, respectively, at 0, 10, and 20 mo postmenarcheal age. The calcium intake required to achieve maximal retention did not change with postmenarcheal age.

**DISCUSSION**

The calcium intake that produces maximal calcium retention in bone is a reasonable criterion for determining calcium requirements. Calcium retention during adolescence can be measured with two techniques. Bone densitometry can determine effects of calcium intake on bone calcium retention over relatively long periods provided calcium intake is maintained. On the other hand, 2- or 3-wk balance studies measure changes in bone calcium indirectly because of the rapid adaptation of balance to dietary changes and because 99% of the body's calcium is in the skeleton and teeth.

In our study the relation between dietary calcium and calcium retention was best described by a nonlinear-regression
model and was asymptotic. Mean (± SEM) maximal skeletal calcium retention was 473 ± 114 mg/d.

At the 1989 RDA of 1200 mg Ca/d for adolescents, only 57% (95% CI: 25%, 89%) of mean maximal calcium retention would be achieved in females aged 12–15 y. A calcium intake of 1500 mg/d, the upper level recommended by the NIH Consensus Panel (11), resulted in 74% (95% CI: 32%, 115%) of mean maximal calcium retention. Because an intake of 1500 mg Ca/d included 100% of mean maximal calcium retention in its CI, we conclude that this intake produces a mean retention that is not distinguishable from the mean maximal retention in this study. A calcium intake of 1300 mg/d was the smallest intake that included 100% of the mean maximal calcium retention in its CI. Therefore, we conclude that to achieve maximal calcium retention in adolescent girls, the daily intake should not be < 1300 mg. This contrasts with the plateau calcium intake of 1500 mg/d for adolescents suggested by others (9). For the first time, our model allows a lower limit (with CI) for dietary calcium intake in adolescents to be established for the goal of achieving maximal skeletal accretion.

Previously, we reported a significant negative relation between postmenarcheal age and calcium retention in adolescents studied at one level of calcium intake (13). When postmenarcheal age was included in our model of the relation between calcium intake and calcium balance, maximal calcium retention decreased with postmenarcheal age. However, the intake required to achieve maximal retention remained unchanged. Other major factors that may affect maximal retention are sex and race. Minor factors such as digestive conditions would also contribute to the variance.

The high intrasubject correlation of calcium balance (r = 0.63), urinary calcium excretion (r = 0.57), and fecal calcium excretion (r = 0.97) at two calcium intakes confirms that the intrasubject consistency reported previously in adults (22) is also present in children. In previous studies we found that urinary calcium excretion was largely independent of calcium intake during adolescence (23). However, in a large cross-sectional evaluation of 381 females aged 8–13 y, a small positive relation (r = 0.212, P < 0.05) between urinary calcium excretion and dietary calcium intake was observed (25). This relation was similar to that in the present study (r = 0.245, P < 0.05), which used a crossover design. Urinary calcium excretion accounted for 9 ± 4% of total calcium excretion.

Evidence from calcium-supplementation trials in children and adolescents is inconclusive regarding the optimal calcium intake for maximizing gain in bone mineral density and subsequent attainment of peak bone mass. All previous trials (5–8).

### TABLE 3

Calcium retention in relation to calcium intake

<table>
<thead>
<tr>
<th>Calcium intake (mg/d)</th>
<th>Percentage of predicted maximal calcium retention</th>
</tr>
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<tbody>
<tr>
<td>800</td>
<td>32 (17, 47)</td>
</tr>
<tr>
<td>900</td>
<td>38 (21, 55)</td>
</tr>
<tr>
<td>1000</td>
<td>44 (23, 65)</td>
</tr>
<tr>
<td>1100</td>
<td>50 (24, 77)</td>
</tr>
<tr>
<td>1200</td>
<td>57 (25, 89)</td>
</tr>
<tr>
<td>1300</td>
<td>63 (26, 100)</td>
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<tr>
<td>1400</td>
<td>69 (29, 108)</td>
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<tr>
<td>1500</td>
<td>74 (32, 115)</td>
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<tr>
<td>1600</td>
<td>78 (37, 119)</td>
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<tr>
<td>1700</td>
<td>82 (43, 122)</td>
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<tr>
<td>1800</td>
<td>86 (49, 122)</td>
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<tr>
<td>1900</td>
<td>89 (55, 122)</td>
</tr>
<tr>
<td>2000</td>
<td>91 (61, 121)</td>
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<tr>
<td>2100</td>
<td>93 (67, 119)</td>
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*7; 95% CIs in parentheses.*
used only two calcium intakes and were of insufficient duration for subjects to attain peak bone mass. The evidence generally showed an advantage of calcium supplementation during puberty. The twin study conducted by Johnston et al (5) indicated that a calcium intake of 1612 mg/d in prepubertal but not in postpubertal subjects resulted in significantly higher gains in spine and radius bone mineral density in one twin compared with the twin who consumed an average of 908 mg/d.

On the other hand, an 18-mo calcium-supplementation trial in 7-y-old Chinese children (n = 84) with a mean calcium intake of 567 mg/d indicated that subjects who received supplementation (with calcium carbonate) to 800 mg Ca/d had significantly greater gains in lumbar spinal bone mineral content than control subjects (7). Furthermore, a 24-mo calcium-supplementation trial in adolescent females (6) indicated that significantly higher gains in bone mineral density and content can be achieved with ∼1340 mg Ca/d compared with ∼980 mg/d, which was given in the control group. In addition, a 12-mo calcium-supplementation study using dairy products in adolescent girls (9–13 y) resulted in significantly higher increases in lumbar spinal bone mineral content and TBBMC in the supplementation group, in which the girls consumed...
1437 ± 366 mg Ca/d (± SD), compared with the control group, in which 728 ± 321 mg Ca/d was consumed (8). In all these studies the lower calcium intake was < 1300 mg/d, the amount necessary to achieve maximal calcium retention so that increases in calcium intake benefited long-term retention.

Whether increases in bone density achieved by adequate calcium intakes can be maintained until skeletal maturity is reached is unknown. Two studies in which follow-up data were collected in children indicated that after calcium supplementation is withdrawn, gains in bone mineral density acquired as a result of previous calcium supplementation are not maintained (25, 26). The authors concluded that calcium supplementation may need to be continued during adolescence for gains in bone mineral density to be maintained.

This study showed that the relation between calcium intake and calcium retention was asympototic in 35 adolescent females aged 12.7 ± 1.2 y who were studied under the same conditions. Maximal calcium retention was 473 mg/d (95% CI: 245, 701 mg/d) and postmenarcheal age strongly affected calcium retention. Minimal dietary calcium intake to achieve mean maximal retention was 1300 mg/d and retention continued to improve with intakes > 2 g/d. Studies are needed to determine dietary and other factors that can shift the curve describing the relation between calcium intake and maximal calcium retention.

REFERENCES