Calcium supplementation provides an extended window of opportunity for bone mass accretion after menarche

Geila S Rozen, Gad Rennert, Roni P Dodiu-Gad, Hedy S Rennert, Nathan Ish-Shalom, Gissel Diab, Batia Raz, and Sofia Ish-Shalom

ABSTRACT

Background: High calcium intakes during adolescence may increase bone acquisition. The magnitude of the effect of dietary calcium supplementation and the timing of its administration to achieve significant effects on bone health are still incompletely defined.

Objective: The objective of this study was to assess the effect of calcium supplementation on bone mass accretion in postmenarcheal adolescent girls with low calcium intakes.

Design: A double-blind, placebo-controlled calcium supplementation study was implemented. One hundred girls with a mean (± SD) age of 14 ± 0.5 y with habitual calcium intakes < 800 mg/d completed a 12-mo protocol. The treatment group received a daily supplement containing 1000 mg elemental calcium. Bone mineral density (BMD) and bone mineral content (BMC) of the total body, lumbar spine, and femoral neck were determined at inclusion, 6 mo, and 12 mo. Also measured were serum concentrations of biochemical markers of bone turnover (osteocalcin and deoxypyridinoline), parathyroid hormone, and vitamin D.

Results: The calcium-supplemented group had greater accretion of total-body BMD and lumbar spine BMD but not BMC than did the control group. Calcium supplementation appeared selectively beneficial for girls who were 2 y postmenarcheal. Calcium supplementation significantly decreased bone turnover and decreased serum parathyroid hormone concentrations.

Conclusion: Calcium supplementation of postmenarcheal girls with low calcium intakes enhances bone mineral acquisition, especially in girls > 2 y past the onset of menarche.

KEY WORDS Calcium supplementation, double-blind study, adolescents, bone density, postmenarcheal girls

INTRODUCTION

As the average life span increases, osteoporosis is a growing health concern in the Western world. Women are at higher risk than men of developing osteoporosis as a result of naturally lower peak bone mass and rapid bone loss after menopause. The results of previous studies have proven that a high adult peak bone mass is protective against late-life fragility fractures (1–4). Nutrition, in particular adequate calcium intake, is one of the major environmental factors believed to have a positive effect on bone accretion, within the limits of genetic boundaries (5–14). In recent years, several studies have reported a positive effect of calcium supple-
tables provided by the same manufacturer. Trained dietitians supplied the tablets monthly.

Follow-up included a monthly interview with a trained dietitian to determine dietary calcium intake. Medical history and menstrual periods were recorded. Compliance was determined by monthly pill count.

**Bone status evaluation**

Bone mineral density (BMD) and bone mineral content (BMC) were measured at the total-body, lumbar spine (LS), and femoral neck (FN) sites by dual-energy X-ray absorptiometry (Lunar DPX scanner; Lunar Corp, Madison, WI). The precision error in vivo was 0.6%, 0.9%, and 1.5%, respectively, for the spine scans (L2–L4) at slow, medium, and fast speeds, whereas the error was 1.2% and 1.5–2.0%, respectively, for the femur scans at slow and medium speeds. The precision of total-body bone density was 0.5% in vitro and in vivo (29, 30). The CV of the BMD measurement at these sites (as determined in young, healthy adults) is between 1% and 1.6%. The scans were acquired by using the appropriate scan mode for the patient’s weight. The same technician performed all measurements. Daily quality-control and phantom measurements were performed to ensure the stability of the equipment during the study period.

**Biochemical markers of bone turnover and calcium-regulating hormones**

Bone-specific alkaline phosphatase was assayed by immunoradiometric assay (Tandem-R-Ostase; Beckman Coulter, Fullerton, CA). Urinary deoxypyridinoline cross-links were evaluated in the second void collected in the morning after the subjects had fasted overnight by use of the Pyrilinks-D enzyme-linked immunosorbent assay (Metra Biosystems, Mountain View, CA). Intact parathyroid hormone (PTH) was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), and 25-hydroxyvitamin D3 was measured by 125I radioimmunoassay (DiaSorin, Stillwater, MN). Osteocalcin was assessed in serum collected in the morning after the subjects had fasted overnight by use of the radioimmunoassay method (OSTK-PR kit; CIS BioInternational, Paris) (31–34).

**Weight and height**

The weight of the girls was measured while they were wearing minimal clothing and no shoes. Weight was recorded to the nearest 0.10 kg, and standing height was recorded to the nearest 0.10 cm. Weights and heights were evaluated by the same operator using the same equipment throughout the study periods. All tests were performed 3 times: at enrollment and after 6 and 12 mo.

**Statistical analysis**

All parameters except for femur BMC had normal distributions according to the Kolmogorov-Smirnov test of normality. However, because of a few outliers, we preferred using nonparametric analyses. The Mann-Whitney U test was used for comparisons of percentage increase and absolute increase in bone measurements between the study and the control groups. Otherwise, a two-tailed Student’s t test was used for comparison of means. A two-factor repeated-measures analysis of variance was used to assess interactions between variables over time. A multivariate analysis using linear regression was used to test the potential effects of different variables on the prediction of percentage gains in BMD and BMC in the different skeletal areas. All results are given as means ± SEMs. The level of significance for all tests was P < 0.05.

**RESULTS**

One hundred girls (76 Jewish girls and 24 Arab girls) completed the 12-mo intervention. The characteristics of the CS and control groups at the time of inclusion to the intervention study are shown in Table 1. At this time, there were no significant differences between the CS and control groups in age, body mass index, height, weight, time since menarche, calcium intake, energy intake, biochemical markers of bone turnover and calcium-regulating hormones, or BMD and BMC of the LS (L2–L4), FN, and total body. No significant change in anthropometric variables (weight and height) was observed during the study period within and between the CS and placebo groups.

**Compliance with treatment**

Twelve girls dropped out of the study. When all of the variables shown in Table 1 were examined, there were no significant differences between the 12 girls who did not complete the study year and the 100 who did. The reasons for dropping out varied.

During the calcium supplementation trial, compliance with
had a calcium intake of 120 mg/d for the placebo group. Only 23.3% of the CS group (3.80 mg/d) compared with 3.07 mg/d for the CS group and 480 mg/d for the placebo group) had a calcium intake > 1300 mg/d.

### Effect of calcium supplementation on bone mass

At the end of the study year, the accretion of total-body BMD was higher in the CS group than in the control group (3.80 ± 0.3% compared with 3.07 ± 0.29%; Table 2). The percentage accretion of BMD in the LS (L2–L4) was higher in the CS group than in the placebo group (3.66 ± 0.35% compared with 3.00 ± 0.43%). The percentage accretion of BMD in the FN tended to be higher in the CS group than in the placebo group, but this difference was not significant.

The accretion of LS BMC was significantly higher in the CS group than in the placebo group after 6 mo (3.53 ± 0.45% compared with 2.20 ± 0.39%), but the differences were no longer significant by the end of the intervention (Table 3).

### Effect of calcium supplementation on serum hormone concentrations and markers of bone turnover

Serum PTH concentrations dropped significantly in the CS group after 6 mo of treatment, by 4.40 pg/mL, compared with an increase in the placebo group of 2.30 pg/mL. This difference was no longer significant after 12 mo (decrease of 1.98 pg/mL in the CS group compared with an increase of 2.96 pg/mL in the placebo group).

Bone turnover as determined by serum osteocalcin concentrations was reduced significantly in the CS group after 6 mo of treatment (a decrease of 1.78 ng/mL when calculated from absolute values; *P < 0.001*), but did not change significantly in the placebo group (an increase of 0.19 ng/mL; Table 4).

Concentrations of bone-specific alkaline phosphatase also dropped significantly in the CS group after 6 mo of treatment (by 4.27 ng/mL compared with 2.07 ng/mL in the placebo group) and even more so by the end of the study year (by 7.10 ng/mL compared with 4.58 ng/mL in the placebo group). Urinary concentrations of deoxypyridinoline cross-links dropped in both groups, and serum concentrations of 25-hydroxyvitamin D$_3$ changed according to season but were not affected by group (data not shown).

### Effect of calcium supplementation and time since menarche on bone mass and bone turnover

There was a significant interaction between time since menarche and treatment group on total-body mass accretion at the end of the study period (*P = 0.014*). When the group was divided by time since the onset of menarche [i.e., by postmenarcheal age (PMA)], 1 y of calcium supplementation had no benefit for girls in the ≤ 24 mo PMA group (total-body BMD of 4.1 ± 2.1% in the CS group compared with 4.3 ± 1.5% in the placebo group; NS) but was very beneficial for girls in the > 24 mo PMA group (total-body BMD of 3.8 ± 1.9% in the CS group compared with 2.1 ± 1.8% in the placebo group; *P = 0.003*; Figure 1). A similar, though nonsignificant, trend was observed for LS BMC.

Repeated-measures analysis of serum osteocalcin concentrations at inclusion and 12 mo showed a significant interaction between time and age since menarche (P = 0.015) but no significant interaction between group and age since menarche. Bone turnover was significantly different as determined by results for serum osteocalcin concentration at inclusion (14.3 ± 0.47 ng/mL compared with 12.5 ± 0.29 ng/mL in girls ≤ 24 mo PMA compared with > 24 mo PMA, respectively; *P = 0.003*) but were almost equal at 12 mo. This difference can be explained by the increase in time since menarche in the ≤ 24 mo PMA group by the end of the year and was not related to treatment. A multivariate regression model showed only time since menarche and treatment group to be significant for the BMD and BMC changes found.

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### Table 2

<table>
<thead>
<tr>
<th>Measurement site</th>
<th>Placebo group (n = 51)</th>
<th>Calcium-supplemented group (n = 49)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6 mo</td>
<td>1.73 ± 0.29</td>
<td>2.23 ± 0.35</td>
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<tr>
<td>12 mo</td>
<td>3.07 ± 0.29</td>
<td>3.80 ± 0.30</td>
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<td>Lumbar spine (L2–L4)</td>
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<tr>
<td>6 mo</td>
<td>1.89 ± 0.32</td>
<td>2.82 ± 0.28</td>
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<tr>
<td>12 mo</td>
<td>3.00 ± 0.43</td>
<td>3.66 ± 0.35</td>
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<tr>
<td>Femoral neck</td>
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<tr>
<td>6 mo</td>
<td>0.44 ± 0.39</td>
<td>1.13 ± 0.41</td>
<td></td>
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<tr>
<td>12 mo</td>
<td>1.39 ± 0.42</td>
<td>2.00 ± 0.51</td>
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</tbody>
</table>

*1 x ± SEM. Percentage accretion refers to the percentage accretion of the bone mass measurements since the time of inclusion in the study, n = 100.

**Significantly different from placebo group (Mann-Whitney U test):**

$^1$P = 0.005, $^2$P = 0.008.

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### Table 3

<table>
<thead>
<tr>
<th>Measurement site</th>
<th>Placebo group (n = 51)</th>
<th>Calcium-supplemented group (n = 49)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body</td>
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<tr>
<td>6 mo</td>
<td>2.45 ± 0.47</td>
<td>2.84 ± 0.44</td>
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</tr>
<tr>
<td>12 mo</td>
<td>4.65 ± 0.54</td>
<td>4.63 ± 0.42</td>
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<tr>
<td>Lumbar spine (L2–L4)</td>
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<tr>
<td>6 mo</td>
<td>2.20 ± 0.39</td>
<td>3.53 ± 0.45</td>
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<tr>
<td>12 mo</td>
<td>3.95 ± 0.58</td>
<td>4.52 ± 0.48</td>
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<tr>
<td>Femoral neck</td>
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</tr>
<tr>
<td>6 mo</td>
<td>1.69 ± 0.83</td>
<td>1.99 ± 0.68</td>
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<tr>
<td>12 mo</td>
<td>3.00 ± 0.81</td>
<td>4.30 ± 0.86</td>
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*1 x ± SEM. Percentage accretion refers to the percentage accretion of the bone mass measurements since the time of inclusion in the study, n = 100.

**Significantly different from placebo group, P = 0.035 (Mann-Whitney U test):**

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TABLE 4
Comparison of plasma parathyroid hormone (PTH), osteocalcin, and bone-specific alkaline phosphatase (BAP) concentrations between the calcium-supplemented and placebo groups throughout the trial.

<table>
<thead>
<tr>
<th></th>
<th>Calcium-supplemented group (n = 49)</th>
<th>Placebo group (n = 51)</th>
<th>p²</th>
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<tr>
<td>PTH (pg/mL)</td>
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<tr>
<td>Baseline</td>
<td>31.4 ± 1.94</td>
<td>30.1 ± 1.47</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute difference between baseline and 6 mo</td>
<td>−4.40 ± 1.96</td>
<td>2.30 ± 1.73</td>
<td>0.012</td>
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<td>Absolute difference between baseline and 12 mo</td>
<td>−1.98 ± 2.06</td>
<td>2.96 ± 1.78</td>
<td>0.07</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.4 ± 0.44</td>
<td>12.9 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute difference between baseline and 6 mo</td>
<td>−1.78 ± 0.39</td>
<td>0.19 ± 0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absolute difference between baseline and 12 mo</td>
<td>−1.35 ± 0.51</td>
<td>−0.39 ± 0.57</td>
<td>NS</td>
</tr>
<tr>
<td>BAP (ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>24.7 ± 1.39</td>
<td>23.4 ± 1.39</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute difference between baseline and 6 mo</td>
<td>−4.27 ± 0.83</td>
<td>−2.07 ± 0.85</td>
<td>0.07</td>
</tr>
<tr>
<td>Absolute difference between baseline and 12 mo</td>
<td>−7.10 ± 0.91</td>
<td>−4.58 ± 0.86</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* ± SEM.
² A two-factor repeated-measures ANOVA with interaction was performed by using the absolute values of each of the 3 variables. In each model, a significant interaction was found between time and group (P < 0.05); therefore, the groups are compared at each point in time by using Student’s t test.

Regression model

A multivariate analysis using linear regression was conducted to determine the most important parameters affecting change in BMD during the intervention year. The model included change in weight and height during the intervention period, group (CS or placebo), change in bone turnover as determined by osteocalcin and deoxypyridinoline cross-links, change in serum PTH concentrations, PMA, ethnic group, and other demographic variables. The most dominant variants positively influencing change in BMD were change in weight during the research year and BMD at the time of inclusion in the study. Calcium supplementation was significant in most models, as was PMA. Markers of bone turnover and calcium-regulating hormones were not significant predictors of change in BMD during the intervention period.

FIGURE 1. Mean (± SEM) percentage gains in total-body bone mineral density during 1 y of treatment with calcium supplementation (○) or placebo (△) by time since menarche. In the group ≤ 24 mo postmenstrual age, n = 25 in the supplemented group and 28 in the placebo group; in the group > 24 mo postmenstrual age, n = 28 girls in the supplemented group and 24 girls in the placebo group. *Significantly different from the placebo group, P = 0.003. There was a significant interaction between time since menarche and treatment group on total-body mass accretion at the end of the study period, P = 0.014 (Mann-Whitney U test).

DISCUSSION

To the best of our knowledge, the present study is the first randomized controlled supplementation trial in adolescent postmenarcheal girls consuming a diet naturally low in calcium. The results of this study show that calcium supplementation significantly enhanced bone acquisition at the LS and the total body after 6 mo; after 12 mo, significant increases were observed for BMD but not for BMC. The percentage gain in bone mass was less pronounced in our study than in previously reported calcium supplementation trials in younger age groups (15–22). A possible explanation of this difference is that the subjects of the present study were adolescent, postmenarcheal girls, whereas in most other studies the subjects were prepubertal or were undergoing puberty, a time of a natural growth spurt and therefore more pronounced bone gain. The fact that BMC measurements were no longer significant after 12 mo may be related to the significant decline in compliance with treatment. Another possible reason for this result is the habitually low calcium intakes of this group; even with the supplementation, some girls in the CS group were not receiving adequate amounts of calcium to accommodate their growth potential during rapid skeletal modeling.

During the first 6 mo of the intervention study, there was a significant reduction in serum PTH, bone-specific alkaline phosphatase, and serum osteocalcin concentrations in the CS group. Previous reports showed that a positive effect on bone mass was achieved when bone turnover declined (35–37). In an intervention study of calcium supplementation in subjects with low calcium intake, a decline in bone turnover was discussed as a possible mechanism of bone gain (18). In our study, however, we believe that the bone gain was not due to a reduction in bone turnover for 2 reasons: 1) the significant reduction in PTH and serum osteocalcin concentrations was cancelled out by the end of the study year and 2) a multivariate model ruled out the change in PTH and serum osteocalcin concentrations as variables affecting change in bone mass.
An unexpected result of our research was that calcium supplementation was more beneficial to girls with longer a PMA (> 24 mo postmenarcheal) than for girls with a shorter PMA (≤ 24 mo postmenarcheal). Even though girls with a longer PMA had a natural deceleration in bone turnover (because they were further from the growth spurt of puberty), calcium supplementation elevated bone gain to a level equal to that of the group with a shorter PMA. Calcium supplementation acted to decrease the deceleration of bone accretion that occurs with increase in age and to create a difference between the CS and control groups that was similar to the natural difference occurring with age, as seen in the control group when comparing girls by PMA. This difference existed even though the girls who were > 24 mo postmenarcheal were significantly older (mean age of 15.2 ± 0.59 y in girls > 24 mo postmenarcheal compared with 14.6 ± 0.64 y in girls ≤ 24 mo postmenarcheal; P = 0.0001) and had less chance of bone accretion as the result of the natural decrease in bone turnover.

We believe that the effect of the total calcium dose consumed by the girls with initially low calcium intakes was more beneficial for the girls with lower bone turnover and consequently lower calcium requirements (with a higher PMA) than for girls with an earlier PMA. These results are encouraging clinically and indicate that there is an extended window of opportunity for bone acquisition than previously reported or questioned (23, 26, 38–41). This finding, although in contrast with the results of other studies, supports observations from a longitudinal study that showed a positive effect of calcium on bone gain during the third decade of life (42). This may have significant clinical implications. For example, calcium supplementation in cases such as late recovery from adolescent anorexia nervosa may prove beneficial to bone health (43, 44).

In a multivariate regression model, calcium supplementation was a significant factor influencing bone acquisition. According to our calculations, the positive effect of calcium supplementation on bone accretion was achieved at an average calcium intake of 1200 mg/d; 500 mg from the low-calcium diet plus 70% compliance with the supplement, equal to 700 mg Ca. This finding supports the new recommendations for calcium intake in adolescent diets (45) and is supported by other publications (6, 23).

In conclusion, the association between calcium intake and bone health at all ages is well established, yet a positive effect on bone accretion is believed to be limited to the early stages of sexual maturation, especially in girls. A unique, not yet fully explained finding in our study was the benefit of calcium supplementation on bone mass accretion in girls ≥ 2 y past the onset of menarche.

SI-S designed the study, obtained the research grant support from the Chief Scientist Fund of the Israeli Ministry of Health, and supervised GSR’s work and the writing of the manuscript. GSR did field research work for the project and writing as part of the requirements for a doctoral thesis. GR participated in the supervision of the research and statistical analysis. HSR performed the statistical analysis. The follow up for the Arabic speaking group was performed by GD. All laboratory work was performed by BR. RPD-G contributed to the fieldwork in the study, and NI-S performed the pediatric follow-up for the study group. None of the authors had any financial or personal interest in this research. All work was purely based on academic interest.

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