

# Nutritional Influences on Bone Growth in Children

## Nutrition Influences Skeletal Development from Childhood to Adulthood: a Study of Hip, Spine, and Forearm in Adolescent Females<sup>1,2</sup>

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**ABSTRACT** This study evaluated the long-term efficacy of supplemental calcium and dairy products on bone mineral areal density of the hip and spine and on the bone geometry and volumetric bone mineral density of the forearm in young females during late adolescence. The study was conducted among participants of a randomized double-blinded, placebo-controlled clinical trial with calcium supplements and among participants of an observational study with higher consumption of dairy products. Hip and spine measurements by dual-energy X-ray absorptiometry were done every 6 mo (dairy group every 12 mo) during last 3 y of the follow-up while peripheral quantitative computerized tomography of the forearm was done at the last visit. The results of the study show a positive influence of calcium supplementation and dairy products on bone mineral density of the hip and the forearm. Dairy products were also associated with a higher bone mineral density of the spine while calcium supplementation did not have an effect. Calcium exerts its action on bone accretion during growth primarily by influencing volumetric bone mineral density while milk may have an additional impact on bone growth and periosteal bone expansion. *J. Nutr.* 134: 701S–705S, 2004.

**KEY WORDS:** • calcium • adolescence • peak bone mass • bone growth

Calcium and proteins may be important determinants of peak bone mass in young adults by influencing bone accretion during growth (1). Adolescence in particular may be a critical period when inadequate calcium nutrition is detrimental to skeletal maturation as calcium requirements are highest during this period of life (2). To understand further potential nutritional determinants of peak bone mass, we conducted a randomized clinical trial with calcium supplementation in a group of young females; in addition, we followed a cohort of young females accustomed to a higher intake of dairy products as a part of an observational study (3). Both studies extended from childhood to young adulthood. Here we report data for several skeletal regions of interest measured during late adolescence.

### MATERIALS AND METHODS

The hip, spine, and forearm measurements were obtained from a group of healthy Caucasian young females who were followed from an average age of 15 to 18 y. One cohort participated in a long-term, double-blinded, placebo-controlled clinical trial with calcium supplementation, and the other participated in an observational study with higher calcium intake from dairy products. The whole study was originally planned for 4 y to cover pubertal growth spurt and was subsequently extended for 3 more years into late adolescence. At the beginning, subjects were recruited from school districts in central Ohio based on the response to the food frequency questionnaire (calcium intake below and above the threshold of 1480 mg/d) (4) and a pubertal stage self-report form. Only individuals in pubertal stage 2 were selected; average chronological age at baseline was  $10.8 \pm 0.8$  y. Subjects with calcium intake below the threshold were recruited into the randomized clinical trial while those with calcium intake above the threshold were allocated to the observational study running parallel to the clinical trial (3). All minors and their parents gave informed consent according to guidelines of the Biomedical Sciences Institutional Review Board at The Ohio State University.

Participants of the clinical trial were randomly assigned to receive either calcium citrate-malate (CCM)<sup>4</sup> supplementation (1000 mg/4 pills/d) or placebo. Procter & Gamble Company provided CCM and placebo pills. Subjects were given a 6-mo supply of pills at each visit;

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<sup>4</sup> Abbreviations used: AP, anterior posterior; BMD, bone mineral density; CCM, calcium citrate-malate; DXA, dual X-ray absorptiometry; pQCT, peripheral Quantitative Computed Tomography.

additional pills were mailed for delayed appointments. Subjects were instructed to take 2 pills in the AM and 2 pills in the PM. Compliance was monitored by pill counts. Participants in the observational component of the study did not receive any intervention, but their dietary habits were monitored; milk was the main source of dietary calcium in the group. There were a maximum of 15 visits (semiannual measurements) for the clinical trial participants and 8 visits (annual measurements) for the dairy group over the 7-y period. By age 15 y there were 103 subjects in the calcium group, 123 in the placebo group, and 88 in the dairy group.

Bone mineral areal density of the hip and spine were measured during the last 3 y and forearm by peripheral Quantitative Computed Tomography (pQCT) at the end of the study (data reported here) while total body and forearm bone mineral areal density, as well as metacarpal radiogrammetry, was measured over the 7-y period (3). Bone mineral areal density measurements were done by dual X-ray absorptiometry (DXA) (1.3q software, GE-Lunar DPX-L). Volumetric bone mineral density measurements of the nondominant radius at the proximal sites (33%) were performed using a pQCT (Norland-Stratec XCT 2000) densitometer with contour, peel, and separation modes of 2,2,2. Nutritional status was determined from 3-d dietary food records using Nutritionist III, v8.5 (Hearst). Total calcium intake in the supplemented group included dietary calcium plus pill calcium adjusted for compliance.

This long-term study allowed us to evaluate the effectiveness of calcium supplementation and dairy products on the bone accretion until the time when most of the bone mass had been accumulated. Pragmatic intent-to-treat analyses were first conducted to measure the effectiveness of calcium supplementation, irrespective of compliance. However, this approach is not without controversy; as compliance decreases, treatment effectiveness decreases, regardless of its efficacy (5,6). Biologic efficacy of calcium was therefore evaluated in a subgroup analysis according to post-hoc stratification based on the average total cumulative calcium intake over time (above/below the median of 1006 mg/d). This led to the formation of a high calcium intake subgroup and a low calcium intake subgroup.

The comparison between the groups was done by the multiple-response ANOVA. The S-plus 2000 package for Windows, Professional Release 3 (Insightful) and Data Desk version 6.1.1 for Macintosh (Data Description) were used for all the statistical analyses.

## RESULTS

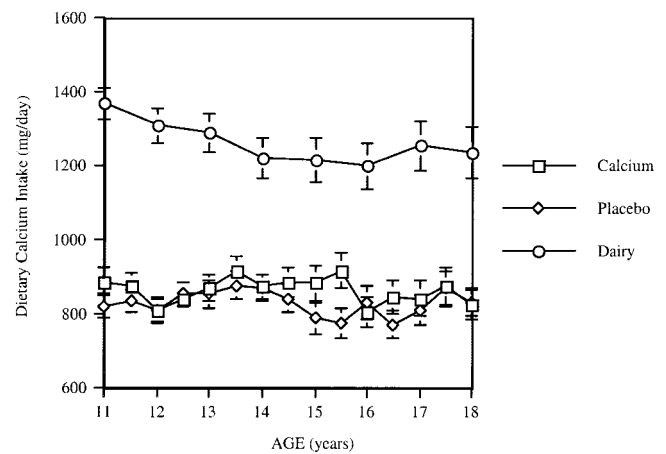
The basic descriptive statistics of young females at an average age 15 y (visit 9) are presented in **Table 1**. There were no significant differences in age, time since menarche, basic anthropometry, dietary calcium and protein intakes, and compliance with pills between calcium-supplemented and placebo individuals. The average dietary calcium intake among the clinical trial participants was 833 mg/d. This level of dietary

**TABLE 1**

*Descriptive statistics of young females at age 15 (visit 9) who completed 7-year study<sup>1</sup>*

Group	Calcium supplemented	Placebo	Dairy
<i>n</i>	79	100	85
Age, y	15.0 ± 0.1	14.8 ± 0.1	15.1 ± 0.1
TSM, y	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Height, cm	163.4 ± 0.6	163.2 ± 0.6	165.9 ± 0.6*
Weight, kg	58.2 ± 1.1	59.5 ± 1.2	61.6 ± 1.2
Pubertal stage	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
Diet Ca intake, mg/d	881 ± 47	785 ± 41	1213 ± 60**
Protein intake, g/d	63 ± 2	63 ± 2	75 ± 2**
Pill compliance, %	70.5 ± 3.3	69.9 ± 3.1	Not applicable

<sup>1</sup> TSM = time since menarche; Results presented as a mean ± SE; \*  $P < 0.01$ , \*\*  $P < 0.001$  for comparison between the dairy and placebo.



**FIGURE 1** Dietary calcium intake among dairy group, calcium supplemented, and placebo subjects over the 7-y period. Results presented as mean ± SE. Three-day food records were obtained annually from the dairy group and semiannually from the clinical trial participants. Calcium = calcium supplemented  $n = 79$ ; placebo,  $n = 100$ ; dairy = high dairy intake,  $n = 85$ .

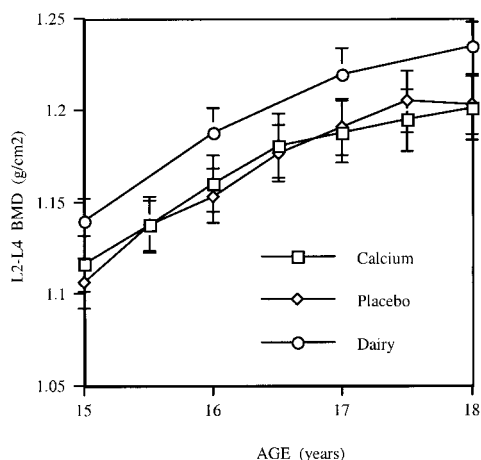
calcium intake remained practically unchanged throughout the entire 7 y period (**Fig. 1**). Based on the reported average compliance with pills of 70.5% of calcium supplemented individuals had total calcium intake of about 1586 mg/d.

Variability in pill compliance resulted in a wide range in total calcium intake in the supplemented group. The post-hoc stratification for subgroups analyses, based on average cumulative total calcium intake (excluding baseline) above and below the median (1006 mg/d), led to the high calcium intake subgroup ( $1494 \pm 292$  mg/d) and low calcium intake subgroup ( $748 \pm 161$  mg/d). The composition of the calcium-supplemented group was similar to that of the high calcium intake subgroup; however, the low calcium intake subgroup was more homogenous.

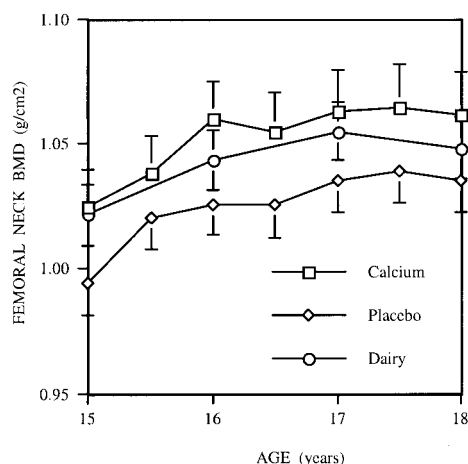
By the average age 15 y, the dairy group subjects remained significantly taller, and had higher dietary calcium and protein intakes as compared to calcium supplemented and placebo groups.

Bone mineral areal density of the anterior posterior (AP) spine ( $L_2$ - $L_4$ ) increased in all three groups from the average age 15 to 18 y (**Fig. 2**). There was no difference in bone mineral density (BMD) of the lumbar spine between the calcium supplemented and the placebo group ( $P = 0.313$ ). However, the dairy group had higher bone mineral density of the spine at age ~15 y, and this was maintained up to the age of ~18 y. Supplemented individuals had significantly ( $P = 0.0024$ ) higher (3%) BMD at femur trochanter (**Fig. 3**); however, at the femoral neck, the difference (1.8%) was not significant ( $P = 0.234$ ) (**Fig. 4**). Bone mineral density of the hip in the dairy group was similar to that of the calcium-supplemented individuals. The above differences were established by age ~15 y and maintained till age ~18 y when BMD at the hip in all three groups started to decline.

Calcium-supplemented individuals had slightly higher volumetric density at the proximal radius ( $1002 \pm 7$  mg/cm<sup>3</sup>) than did the placebo subjects ( $990 \pm 6$  mg/cm<sup>3</sup>) and dairy group participants ( $996 \pm 7$  mg/cm<sup>3</sup>); however, the differences between the groups were not significant ( $P = 0.41$ ) (**Fig. 5**). Contrary to this, the cross-sectional area of the proximal radius was much higher in the dairy group compared to the clinical trial individuals ( $P = 0.008$ ) (**Fig. 6**). Dividing the subjects into subgroups according to the average total cumu-



**FIGURE 2** Bone mineral areal density of the spine (L2-L4) among calcium supplemented subjects (calcium,  $n = 79$ ), placebo subjects (placebo,  $n = 100$ ), and dairy group (dairy,  $n = 85$ ), from according to age. Results presented as mean  $\pm$  SE; no significant differences among study groups by ANOVA.



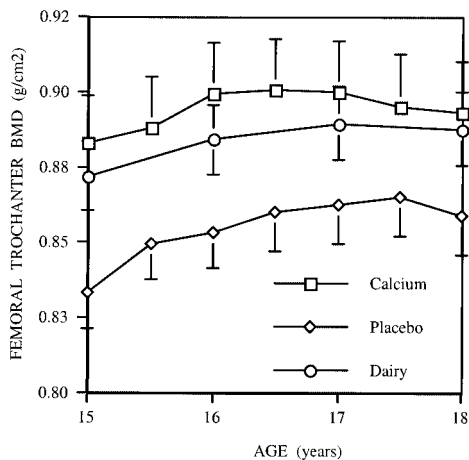
**FIGURE 4** Bone mineral areal density of the femur neck among calcium supplemented subjects (calcium,  $n = 79$ ), placebo subjects (placebo,  $n = 100$ ), and dairy group (dairy,  $n = 85$ ) according to age. Results presented as mean  $\pm$  SE. No significant differences among study groups by ANOVA.

lative calcium intake over time revealed a significantly higher volumetric BMD at the proximal radius in the high calcium intake subgroup ( $1008 \pm 6 \text{ mg/cm}^3$ ) compared to the low calcium intake subgroup ( $982 \pm 6 \text{ mg/cm}^3$ ), with dairy individuals being in between ( $996 \pm 7 \text{ mg/cm}^3$ ) ( $P = 0.018$ ) (Fig. 7). Cortical area of the proximal radius was the highest in the dairy group ( $P = 0.0003$ ) (Fig. 8), while cortical to total area ratio was slightly higher in the high calcium intake subgroup ( $P = 0.045$ ) (Fig. 9).

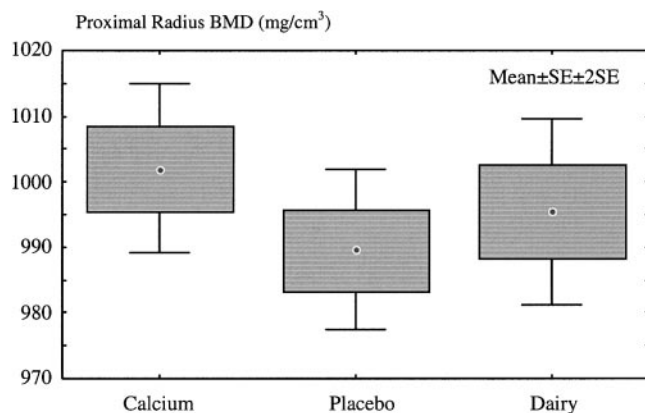
**DISCUSSION**

This study documented that calcium influences bone mass acquisition in the hip and in the forearm. The effect is relatively weak, as the habitual dietary calcium intake in the population under study was in the range of 800 to 900 mg/d and close to the calcium intake threshold for older adolescents and young adults. The observed differences in

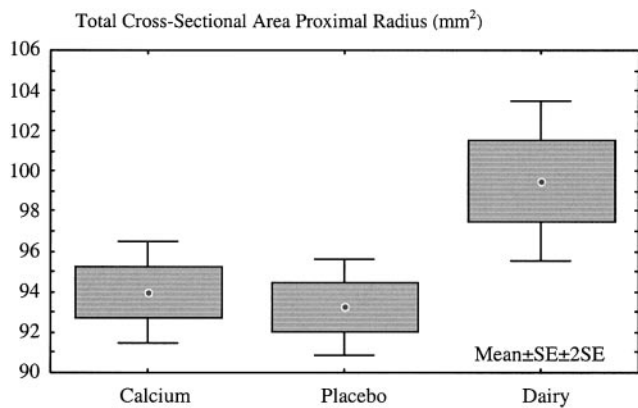
bone mineral density at the above skeletal regions of interest were presumably established earlier during growth and maintained into late adolescence and young adulthood. This probably happened during pubertal growth spurt when extra calcium intake was necessary to accommodate longitudinal bone growth and periosteal bone expansion (3,7). The habitual calcium intake at that level was simply not enough to accommodate growth as well as the endosteal apposition of bone. During pubertal growth spurt, about 37% of the entire skeletal mass is accumulated (8). Therefore, inadequate calcium intake during this period may compromise volumetric bone density as the results of this research indicated. As there were no baseline data for the hip and forearm pQCT measurements, this fact needs to be confirmed through a long-term intervention study. However, as the clinical trial participants were carefully matched at the beginning of the study with all the biological parameters being similar (3), the implication is that the same may apply to the hip and forearm as well. Calcium supplementation on top of the habitual dietary calcium



**FIGURE 3** Bone mineral areal density of the femur trochanter among calcium supplemented subjects (calcium,  $n = 79$ ), placebo subjects (placebo,  $n = 100$ ), and dairy group (dairy,  $n = 85$ ) according to age. Results presented as mean  $\pm$  SE. Calcium supplemented versus placebo,  $P = 0.0024$  across all ages by ANOVA.



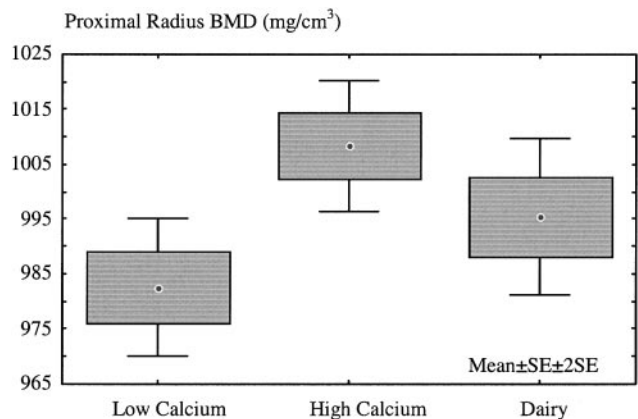
**FIGURE 5** Box-plots of the volumetric bone mineral density of the proximal radius among calcium supplemented subjects (calcium,  $n = 79$ ), placebo subjects (placebo,  $n = 100$ ), and dairy group (dairy,  $n = 85$ ). No significant differences among study groups by ANOVA)



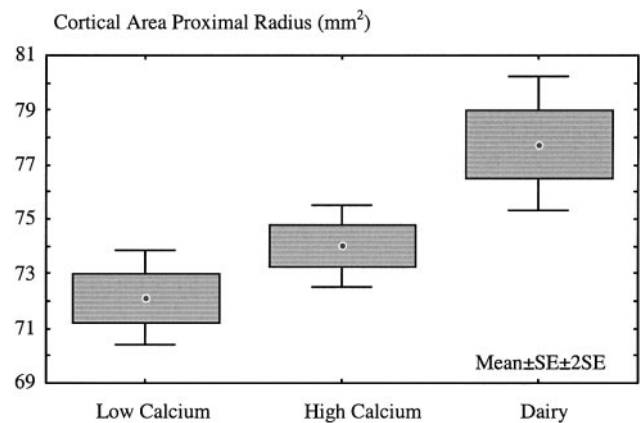
**FIGURE 6** Box-plots of the total cross-sectional area of the proximal radius among calcium supplemented subjects (calcium,  $n = 79$ ), placebo subjects (placebo,  $n = 100$ ), and dairy group (dairy,  $n = 85$ ). Dairy versus calcium or placebo,  $P = 0.008$  by ANOVA.

intake of  $\sim 830$  mg/d did not influence bone mineral areal density of the AP lumbar spine; however, dairy group individuals had much higher density values than did their clinical trial counterparts. The dairy group subjects were taller and had higher bone volume of the radius as presented in the total cross-sectional area. Their calcium intake from dairy products was almost double at baseline, and they had  $\sim 20\%$  higher protein intake in comparison to the subjects selected for the clinical trial. The differences in bone volume between dairy and clinical trial groups could simply be explained due to more favorable impact of milk (due to its mineral and protein content) (9–11) on bone expansion during early growth, childhood years, and/or by a combination of other healthy life-style factors, such as physical exercise, associated with milk-drinking habits (12,13); however, we do not have evidence to support the latter. Periosteal bone expansion, irrespective of density, contributes to bone strength. The above findings are clinically important and indicate a need for an intervention study with dairy and calcium supplements early in childhood.

All clinical studies with calcium or dairy products supplementation in children and adolescents reported positive



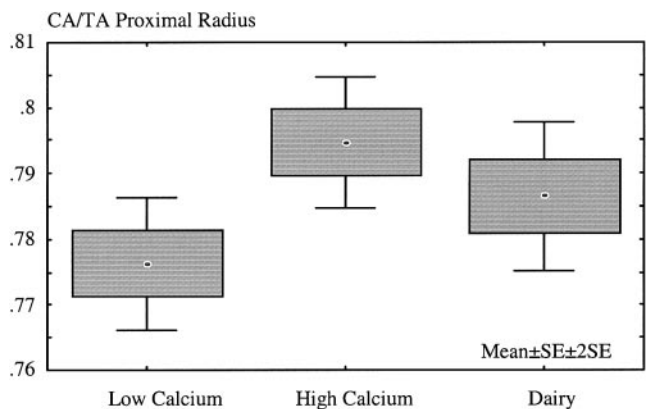
**FIGURE 7** Box-plots of the volumetric bone mineral density of the proximal radius among clinical trial participants stratified according to the average cumulative calcium intake below (low calcium subgroup) and above the median (high calcium subgroup) and the dairy group. (high calcium vs low calcium group,  $P = 0.018$  by ANOVA).



**FIGURE 8** Box-plots of the cortical area of the proximal radius among clinical trial participants stratified according to the average cumulative calcium intake below (low calcium subgroup) and above the median (high calcium subgroup) and the dairy group. (dairy versus high or low calcium group,  $P = 0.0003$  by ANOVA).

effect of intervention on bone mass (14–22). However, all these studies were too brief to address the issue of peak bone mass. The increase in bone mass observed in those studies could be explained to a large extent by the remodeling transient phenomenon (23). In some of the studies reported earlier, the difference in bone mass between the groups diminished after calcium intervention was discontinued (24,25), indicating that the gain in bone accretion was lost as a result of the second transient (23). In other studies, the effects of intervention were maintained (22,26,27). This may be specific to the calcium source and/or the level of habitual dietary calcium intake in the population under study. Given that bone mineral density of the hip and the forearm of young females in this study reached a higher level after a prolonged calcium supplementation, a follow-up study is necessary to resolve this issue.

In conclusion, this study indicated that calcium and dairy products influence bone mass acquisition, leading to a higher peak bone mass. Calcium exerts its action on bone accretion during growth primarily by influencing volumetric bone mineral density, while dairy products may have an



**FIGURE 9** Box-plots of the cortical area/total area (CA/TA) of the proximal radius among clinical trial participants stratified according to the average cumulative calcium intake below (low calcium subgroup) and above the median (high calcium subgroup) ( $P = 0.045$ , by ANOVA) and the dairy group.



additional impact on bone growth and periosteal bone expansion.

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