Calcium supplementation and bone mineral density in females from childhood to young adulthood: a randomized controlled trial[1–3]

Velimir Matkovic, Prem K Goel, Nancy E Badenhop-Stevens, John D Landoll, Bin Li, Jasminka Z Ilich, Mario Skugor, Larry A Nagode, Stacey L Mobley, Eun-Jeong Ha, Thomas N Hangartner, and Albert Clairmont

ABSTRACT

Background: Short-term studies established that calcium influences bone accretion during growth. Whether long-term supplementation influences bone accretion in young adults is not known.

Objective: This study evaluated the long-term effects of calcium supplementation on bone accretion among females from childhood to young adulthood.

Design: A 4-y randomized clinical trial recruited 354 females in pubertal stage 2 and optionally was extended for an additional 3 y. The mean dietary calcium intake of the participants over 7 y was ≈830 mg/d; calcium-supplemented persons received an additional ≈670 mg/d. Primary outcome variables were distal and proximal radius bone mineral density (BMD), total-body BMD (TBBMD), and metacarpal cortical indexes.

Results: Multivariate analyses of the primary outcomes indicated that calcium-supplementation effects vary over time. Follow-up univariate analyses indicated that all primary outcomes were significantly larger in the supplemented group than in the placebo group at the year 4 endpoint. However, at the year 7 endpoint, this effect vanished for TBBMD and distal radius BMD. Longitudinal models for TBBMD and proximal radius BMD, according to the time since menarche, showed a highly significant effect of supplementation during the pubertal growth spurt and a diminishing effect thereafter. Post hoc stratifications by compliance-adjusted total calcium intake and by final stature or metacarpal total cross-sectional area showed that calcium effects depend on compliance and body frame.

Conclusions: Calcium supplementation significantly influenced bone accretion in young females during the pubertal growth spurt. By young adulthood, significant effects remained at metacarpals and at the forearm of tall persons, which indicated that the calcium requirement for growth is associated with skeletal size. These results may be important for both primary prevention of osteoporosis and prevention of bone fragility fractures during growth. *Am J Clin Nutr* 2005;81:175–88.

KEY WORDS Calcium, growth, skeletal development, peak bone mass, osteoporosis, females

INTRODUCTION

Peak bone mass is one of the main determinants of osteoporotic fracture in humans (1); it is strongly influenced by genetics, but it could also be related to nutrition and exercise (2). We suggested previously that calcium might be an important determinant of peak bone mass in young adults by influencing bone accretion during growth (1, 3, 4). All clinical trials with calcium supplements in children and adolescents completed to date were relatively short and showed a positive effect of intervention on bone mass in young persons (3, 5–10). However, the extent to which those benefits could be translated to skeletal maturity was not confirmed. To understand further the nutritional determinants of peak bone mass, we conducted a long-term study with calcium supplementation in a cohort of females from childhood to young adulthood. The 7-y study included phases of bone modeling (change in size and geometry) during the pubertal growth spurt and of bone consolidation (endosteal apposition) during late adolescence, which represent the periods before and after epiphyseal closure, respectively.

SUBJECTS AND METHODS

Participants and sample size

The study was conducted in a cohort of young females recruited from 20 school districts in central Ohio. The minimum sample size calculation, based on the forearm bone mineral density (BMD) measurements in a pilot study (3), showed that 105 subjects per group were required to detect a 2.7% difference in BMD at the proximal radius by the end of the second year at $\alpha = 0.05$, with power ≥ 0.8. An invitation form, a food-frequency

[1] From the Osteoporosis Prevention and Treatment Center, the Bone and Mineral Metabolism Laboratory, and the Departments of Physical Medicine & Rehabilitation, Medicine, and Nutrition, Davis Medical Research Center (VM, NEB-S, JDL, E-JH, and AC) and the Departments of Statistics (PKG and BL) and Veterinary Biosciences (LAN), The Ohio State University, Columbus; the School of Allied Health, University of Connecticut, Storrs (JZI); the Department of Endocrinology, Cleveland Clinic, Cleveland (MS); the Nutrition Care Directorate, Walter Reed Army Medical Center, Washington, DC (SLM); and the Bone Imaging Laboratory, Wright State University, Dayton, OH (TNH).

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[3] Address reprint requests to V Matkovic, Osteoporosis Prevention and Treatment Center & the Bone and Mineral Metabolism Laboratory, Davis Medical Research Center, The Ohio State University, 480 West 9th Avenue, Columbus, OH 43210. E-mail: matkovic.1@osu.edu.

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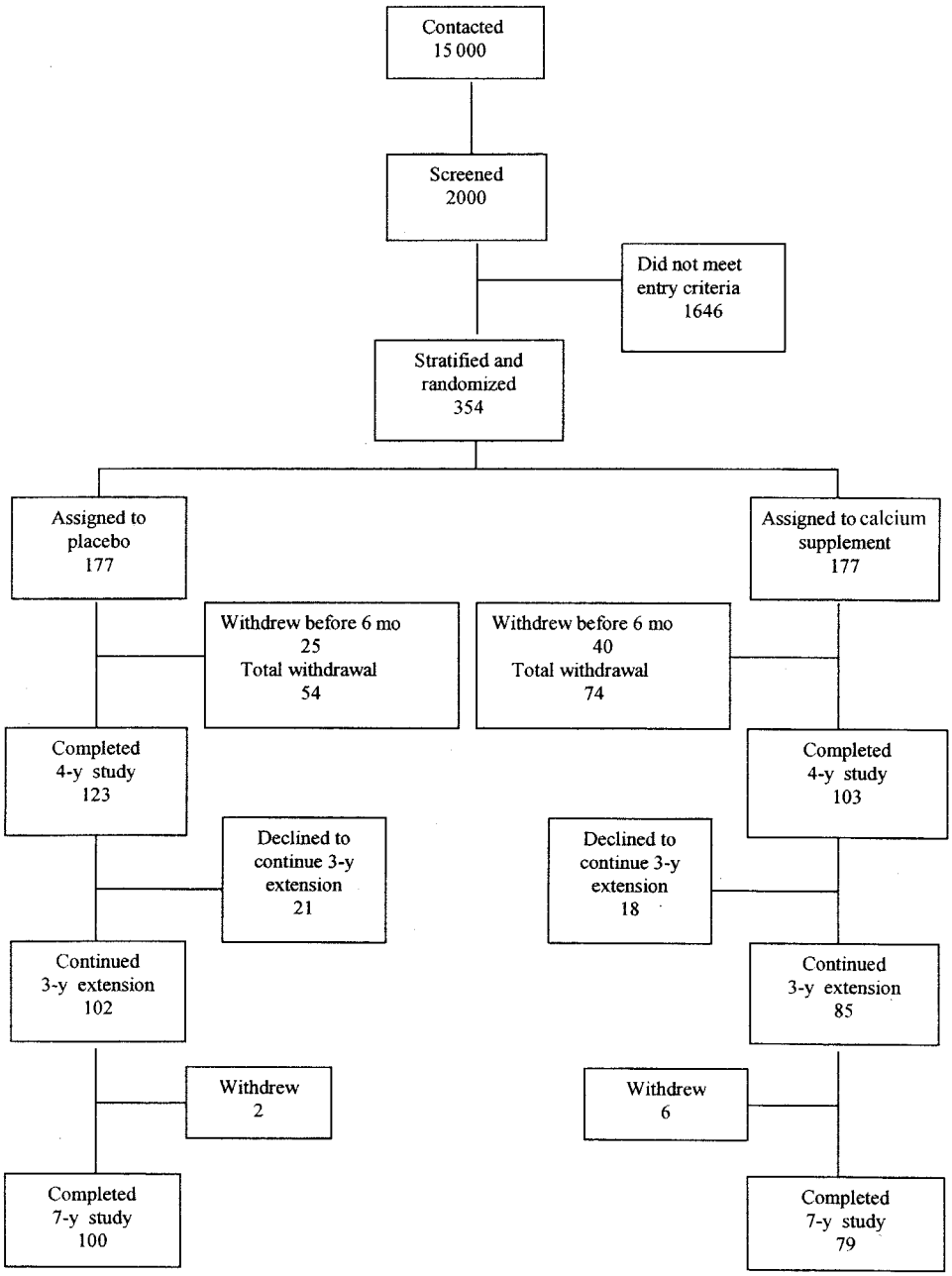

FIGURE 1. Study profile and subject disposition. Within the first 3 mo of the trial, 23.2% of girls from the supplemented group and 14.1% of girls from the placebo group dropped out of the study because of difficulty in swallowing the pills. No reason for the difference in withdrawal between the 2 groups is obvious. Additional disproportionate dropout occurred after year 4, when subjects were given the option of not continuing in the 3-y extension. Fifty-one percent of the subjects completed the 7-y trial. One subject who became morbidly obese and could not complete the dual-energy X-ray absorptiometry measurements remained in the study but was excluded from the analysis.

questionnaire, and a pubertal stage self-report form were mailed to 15 000 female students aged 8–13 y (11). The inclusion criteria were white race, normal physical and mental health, pubertal stage 2, and calcium intake (determined by food frequency) below the threshold level of 1480 mg/d (12). The exclusion criteria were a history of metabolic bone, kidney, liver, or celiac disease; use of oral cortisone, hormones, diuretics, or antiseizure medications; other current systemic, chronic disease; and the presence of clinically significant abnormal laboratory data on screening. Response rates of 8–20% across schools generated initial data on 2000 girls who were willing to participate in the study. From this screening sample, 354 persons satisfied the inclusion criteria and were recruited for the study (**Figure 1**).

The study protocol was approved by the Biomedical Sciences Institutional Review Board at The Ohio State University. All minors and their parents gave written informed consent.

Study protocol

This study was originally designed as a randomized, double-blind, 4-y controlled clinical trial to assess the effect of calcium citrate-malate supplementation (1000 mg/d, given in 4 pills) on

the BMD of the total body, the radius, and the metacarpal radiogrammetric measurements of adolescent females during the pubertal growth spurt. The study was subsequently extended for 3 y, into late adolescence, in the subjects who agreed to continue; double-blind status was preserved. On the basis of our previous cross-sectional study (13), participants were first separated into 4 strata with the use of baseline total-body BMD (TBBMD) and body mass index (BMI; in kg/m^2), each at 2 levels (above and below the average value). The characteristics of the subjects were TBBMD $<$ 0.879 and BMI $<$ 18.5 in stratum A (n = 122), TBBMD $<$ 0.879 and BMI $\geq$ 18.5 in stratum B (n = 45), TBBMD $\geq$ 0.879 and BMI $<$ 18.5 in stratum C (n = 79), and TBBMD $\geq$ 0.879 and BMI $\geq$ 18.5 in stratum D (n = 108). The purpose of stratification was to allow for the equal distribution of bone measurements between the placebo and supplemented groups. Within each stratum, subjects were randomly assigned to either group. A list of consecutive random assignments to the calcium supplementation and placebo groups within each stratum, prepared by a statistician, led to 177 subjects within each group. A simple coding system linked the drug packages to the randomization list. The Proctor & Gamble Company provided calcium citrate-malate and placebo (microcrystalline cellulose) pills whose palatability and appearance were equal. Subjects were given a 6-mo supply of pills at each visit; additional pills were mailed if a return appointment had to be postponed. Subjects were instructed to take 2 pills in the morning and 2 pills in the evening. Compliance was monitored by pill counts and assessed by fecal calcium density, serum parathyroid hormone (PTH) concentrations, and 24-h urinary calcium excretion.

Outcome measures and confounding factors

Primary outcome measures in this study were bone mineral areal density of the whole body and radius at 2 sites [proximal (33% of the radius length) and distal (10% of the radius length)], and metacarpal cortical index. Metacarpal radiogrammetry was previously used in the study of nutrition, peak bone mass, and hip fracture rates in adults (1) and was shown to distinguish children with upper-limb fractures from their control subjects (14). Secondary outcome measures in the current study were stature, bone width, bone area, and bone turnover markers. Measures to control for confounding factors included assessment of nutritional status (ie, dietary calcium, protein, energy intake, and 24-h urinary sodium), energy expenditure, body weight, skeletal age, and pubertal development. Medical history, physical examination, and completed dietary and physical activity questionnaires and weight, height, and bone mass measurements of the whole body and the forearm were obtained at baseline and every 6 mo; blood (drawn between 0800 and 1700), 24-h urine, and stool samples (no baseline data) were obtained at baseline and annually. Hand X-rays for skeletal age and radiogrammetry of the metacarpal bones were obtained at baseline and at years 4 and 7. The subject's weight was measured to the nearest 0.1 kg while the subject was wearing normal indoor clothing but no shoes. Standing height to the nearest 0.1 cm was recorded on a wall-mounted stadiometer (nearest 0.1 cm) while the subject was without shoes and with the mandible plane parallel to the floor (11). The subjects self-assessed their pubertal stage by marking on a chart the appropriate figures of sexual development, and the onset of menarche was documented within 6 mo of the event (15). Body composition (ie, lean body mass and body fat) and BMD were measured by using dual-energy X-ray absorptiometry (DXA; GE-Lunar DPX-L) with DPX-L software (version 1.3q; GE-Lunar). The precision errors (%CV) for the whole body and radius shaft BMD measurements were 0.5% and 0.8%, respectively (15). The two-person interobserver error for DXA analysis was $<$ 0.1%. Daily phantom measurements on the DXA indicated a steady but extremely slow machine drift (a total decrease of 1.8% over 5 y); BMD was adjusted accordingly. Skeletal age was determined from radiographs of the nondominant hand by using the FELS method (16). Radiogrammetry of the metacarpals was performed by using the automated X-Posure System (Pronosco A/S, Vedboek, Denmark; 17). The CVs for the static and repositioned measurements were 0.0% and 0.2% for bone width and 0.5% and 0.6% for cortical thickness, respectively (18). Metacarpal cortical area (CA) and total cross-sectional area (TA) and the metacarpal cortical index, the ratio of CA to TA, were calculated. Nutritional status was assessed from 3-d dietary food records by using NUTRITIONIST III software (version 8.5l; Hearst Corp, San Bruno, CA). Weight-corrected energy expenditure was estimated by recording activity in 15-min intervals for 2 d (11, 19). Serum urine and stool specimens were stored at -80 °C and -20 °C, respectively, and analyzed in multiple batches at different times throughout the study. Basic blood and urine chemistry measurements were made by using a Hitachi 717 Chemistry Analyzer (Boehringer-Mannheim, Indianapolis), and serum calcium was adjusted per total protein. Stool samples were collected before visits and aliquot used for fecal calcium density (mg calcium/g dry feces) measurement as previously described (20). Urine and fecal calcium were measured by atomic absorption spectrophotometry. Urinary *N*-telopeptides (NTX; nmol · L bone collagen equivalent/24 h) were measured by a competitive inhibition enzyme-linked immunosorbent assay (Osteomark; Ostex International, Seattle). Serum 25-hydroxyvitamin D_3 [$25(OH)D_3$] was measured in each sample (excluding baseline) by using a radioimmunoassay (RIA) with ^{125}I-labeled tracer (21). Serum osteocalcin was measured by immunoradiometric assays, and PTH was measured by Allegro immunoassay kits (both: Nichols Institute, San Juan Capistrano, CA). The stability of the PTH was shown by 99.1% agreement between repeated assays of a subsample (n = 10) in 1994 and 2000.

Statistical analysis

This long-term study allowed us to evaluate the effectiveness of calcium supplementation on bone mineral accretion during the period when most of the bone mass is accumulated. The distributions of confounding factors within the calcium-supplemented and placebo groups were compared by using the two-sample Kolmogorov-Smirnov tests for equality of distributions of average cumulative response over all visits for the 4- and 7-y cohorts. For the 7-y cohort, the distributions of average cumulative response over all visits during the first 4 y were also compared.

The primary outcome variables were analyzed by using repeated-measures multivariate analysis of variance (MANOVA) (visits 1–9 or 1–15) consisting of main effects and interactions for treatment and visits, as well as a more general MANOVA (visits 2–9 or 2–15), in which the observations on the same person were treated as response variables with the use of an arbitrary variance-covariance matrix with baseline values as covariates. The gains from baseline were also analyzed by using repeated-measures MANOVA with baseline values as covariates. If the repeated-measures MANOVA indicated a significant interaction

effect or if the general MANOVA indicated significant differences between the mean responses, the follow-up univariate analyses [*t* test for gain and analysis of covariance (ANCOVA), with baseline values as covariates for the placebo and supplemented groups] at the endpoints of the original 4-y and the extended 7-y clinical trial are also presented. All these tests were made at $\alpha = 0.05$. Even though the subjects made ≤15 visits, the mean (± SD) data are shown only for the baseline and the 4-y and 7-y endpoints.

An intent-to-treat analysis was performed to measure the programmatic effectiveness of calcium supplementation while ignoring the lack of full compliance (22, 23). Because the distributions of the confounding factors over time did not differ significantly, we did not include any of these variables in the analysis of primary outcomes. Furthermore, because the baseline measurements for cohorts in the 4-y and 7-y analyses did not differ significantly, no adjustments for differential dropouts were made.

Significant interactions in the repeated-measures MANOVA, as well as the results from the general MANOVA and the follow-up univariate analyses, suggested a possible catch-up phenomenon in bone mass acquisition in the placebo group at some of the skeletal sites. To obtain an insight into the skeletal physiology of bone growth and the timing of the catch-up phenomenon, longitudinal analyses based on the linear mixed-effect model (LME; 24, 25), with years since menarche (YSM) as the time line, group effects as fixed, and subject effects as random, were carried out by using data from subjects for whom there were ≥2 observations and with known onset of menarche ($n = 252$) for some of these skeletal sites.

The YSM is the best determinant of bone physiology in pubertal females. It serves as a biological clock for the events associated with menarche (26–28), and it minimizes the effect of heterogeneity on subjects' bone biology within each visit. The fixed group effects for bone mass accretion patterns in our LME model are represented by regression splines, ie, piece-wise cubic polynomials; constraints are such that the overall function is continuous and smooth at the joining points of these polynomials (knots). We used a natural-spline model (25), with fixed specified knots that further assumes linear trends in segments outside the lower and upper boundary knots. The large-sample chi-square tests for the significance of fixed effects in LME are based on likelihood ratio statistics (25). We also obtained 95% confidence bands for the difference in bone mineral areal density patterns for the corresponding groups. The band provides the estimated range of YSM values during which the patterns being compared differ significantly. Conservatively, when this confidence band contains zero, the difference at that time point is not significant at $\alpha = 0.05$. In addition, because the chi-square test is geared to test multiple hypotheses, it is possible that the LME model *P* value indicates highly significant fixed effects, but the confidence band may show insignificant differences at the full range of YSM time points. This phenomenon is similar to the *F* test in ANOVA, in which the *F* test shows significance, but the pairwise comparisons may not differ significantly.

This modeling strategy allows flexible shapes for regression functions in the analysis of longitudinal data (29–32). Our model assumes that a subject-specific bone-mass accretion pattern is a random effect that differs from its treatment group by an intercept term. It is similar to the model reported earlier in a bone-mass modeling study (32). This model was used to estimate patterns for the primary and secondary outcome variables and calcium metabolic measurements. On the basis of bone biology, 4 knots at −2, 0, +2, and +4 YSM were used in bone outcome models. For the bone turnover markers, serum calcium, serum PTH, and fecal and urinary calcium concentrations, which were collected yearly, 3 knots at −2, 0, and +2 YSM were used. Because the randomization to the 2 treatment groups was performed within each of the 4 strata, our initial model for the 2 × 2 × 2 factorial experiment included distinct regression splines for each subgroup (25). However, the factor "baseline BMI" was dropped from the model because it showed no significant effect on the bone accretion profiles. Furthermore, the factor "baseline TBBMD" had only a significant main effect (± 0.038, $P < 0.001$) in the TBBMD accretion patterns without any interaction with the pill or placebo factor. The models were fitted for different combinations of boundary knots, all of which provided qualitatively similar results. The results based on boundary knots at −3 YSM (baseline average − SD) and at +6 YSM (last visit average + SD) are presented here.

The biologic efficacy (22, 23) of calcium intake was evaluated by using LME analysis of data after post hoc stratification of subjects based on the average total cumulative calcium intake over time (ie, above or below the habitual dietary calcium intake of 830 mg/d), irrespective of assigned group. Total calcium intake for subjects in the supplemented group included dietary calcium plus pill calcium after adjustment for compliance.

Finally, post hoc stratifications according to the final height or the TA above and below the median (ie, tall or short and larger or smaller bones, respectively) were also performed in subgroup analyses to establish whether body or bone size affects calcium requirement as previously indicated (33, 34). The two-factor (ie, group and size) ANCOVA for BMD at proximal radius and cortical bone mass (CA), with the baseline value of the response variable used as a covariate, was performed at both the year 4 and the year 7 endpoints to assess the overall differences among the 4 subgroups by using the *F* test. The contrast between the means of the placebo and calcium-supplemented groups for persons within each size subgroup was tested by using *t* tests to assess their interactions. We used S-PLUS 2000 for WINDOWS software (Professional Release 3; Insightful Corporation, Seattle) for all statistical analyses (35).

RESULTS

Study population

Participant flow is shown in Figure 1. Baseline characteristics of the study population are presented in **Table 1**. There were no significant differences at baseline in any of the values between the placebo and calcium-supplemented groups. Moreover, the cohorts with ≥2 visits, those who completed the 4-y and 7-y intervention, and those in the high or low calcium intake subgroups did not differ significantly in baseline characteristics between the 2 groups. In addition, the distributions of the confounding factors, including pubertal stage, skeletal age, dietary proteins, energy intake and expenditure, and urinary sodium, did not differ significantly between the 2 groups throughout the study (range of *P* values: 0.30–0.83). The average serum calcidiol concentration over time was 27.9 ± 10.2 ng/mL and 27.5 ± 10.9

TABLE 1
Baseline characteristics for subjects in the supplemented and placebo groups[1]

Variables	Supplemented group	Placebo group	P^2
Demographic			
Age (y)	10.9 ± 0.9	10.8 ± 0.7	0.30
Time since menarche (y)	−2.0 ± 1.0	−1.9 ± 1.0	0.58
Pubertal stage (SMI)	1.9 ± 0.5	1.9 ± 0.4	0.81
Skeletal age (y)	11.3 ± 1.1	11.5 ± 1.0	0.32
Anthropometric			
Height (cm)	144.8 ± 7.4	145.2 ± 7.0	0.67
Weight (kg)	39.1 ± 8.0	40.2 ± 9.0	0.21
Lean body mass (kg)	27.4 ± 3.8	27.7 ± 3.9	0.57
Body fat (kg)	9.0 ± 5.2	9.9 ± 6.3	0.15
Percentage body fat (%)	23.4 ± 8.8	24.6 ± 9.9	0.23
Bone			
Total-body BMC (g)	1310 ± 251	1309 ± 234	0.95
Total-body BMD (g/cm^2)	0.892 ± 0.061	0.891 ± 0.059	0.85
Total-body BMA (cm^2)	1460 ± 194	1461 ± 185	0.96
Proximal radius BMC (g)	1.042 ± 152	1.050 ± 0.147	0.63
Proximal radius BMD (g/cm^2)	0.503 ± 0.047	0.502 ± 0.047	0.88
Proximal radius BMA (cm^2)	2.069 ± 0.194	2.089 ± 0.199	0.33
Distal radius BMC (g)	0.627 ± 0.106	0.619 ± 0.106	0.48
Distal radius BMD (g/cm^2)	0.280 ± 0.041	0.277 ± 0.039	0.47
Distal radius BMA (cm^2)	2.244 ± 0.257	2.237 ± 0.241	0.79
Metacarpal total area (cm^2)	0.392 ± 0.056	0.397 ± 0.051	0.37
Metacarpal cortical area (cm^2)	0.137 ± 0.021	0.136 ± 0.017	0.64
Ratio of metacarpal cortical area to total area	0.350 ± 0.036	0.344 ± 0.038	0.12
Nutrition and activity			
Energy intake (kcal/d)	1931 ± 385	1948 ± 401	0.71
Protein intake (g/d)	70 ± 18	70 ± 17	0.90
Calcium intake (mg/d)	855 ± 288	819 ± 302	0.30
Energy expenditure (kcal/d)	1195 ± 469	1285 ± 554	0.14
Blood and urine chemistry			
Serum calcium (mg/dL)	9.4 ± 0.3	9.4 ± 0.3	0.94
Serum total protein (g/dL)	7.1 ± 0.4	7.1 ± 0.3	0.51
Serum osteocalcin (ng/mL)	19.6 ± 4.2	19.5 ± 4.4	0.86
Alkaline phosphatase (IU/L)	307 ± 82	316 ± 75	0.32
Serum PTH (pg/mL)	28.8 ± 13.5	28.2 ± 12.8	0.65
Urinary calcium (mg/d)	89 ± 60	79 ± 50	0.11
Urinary sodium (mg/d)	2546 ± 1039	2443 ± 1042	0.39
Urinary NTX (nmol · L^{-1} · d^{-1})	2488 ± 1088	2460 ± 1422	0.85

[1] All values are $\bar{x}$ ± SD. SMI, sexual maturity index (mean of breast and pubic hair development stage); BMC, bone mineral content; BMD, bone mineral density; BMA, bone mineral area; PTH, parathyroid hormone; NTX, *N*-telopeptide.
[2] Two-sample *t* test for the difference between the placebo (n = 177) and supplemented (n = 177) groups.

ng/mL in the supplemented and placebo groups, respectively (range of *P* values: 0.33–0.55). The placebo group had a slightly higher proportion of ever-users of contraceptives (23%) than did the supplemented group (13%). However at age ≈18 y, the two-way (ie, group and contraceptive use indicator) ANOVA for the bone variables found no interaction between the factors TBBMD ($P = 0.642$), proximal radius BMD ($P = 0.758$), and CA:TA ($P = 0.527$).

The average dietary calcium intake among the study participants was 830 ± 236 mg/d. This intake remained practically unchanged over the 7-y period and was considered the habitual dietary calcium intake of the study population (**Table 2**). The average total calcium intake in the supplemented group (1498 ± 318 mg/d) was close to the calcium intake threshold for adolescents (12). Total calcium intake in the supplemented group declined by ≈10% from age 15.5 y to age 18 y, primarily because of a decrease in compliance with pill taking (Table 2). In addition, several placebo group subjects had an average cumulative calcium intake comparable to the total calcium intake in the supplemented group.

Compliance measures

The average cumulative compliance with pill taking during both the first 4-y period (70 ± 20% and 71 ± 21% for the supplemented and placebo groups, respectively) and the 7-y period (65 ± 22% and 66 ± 22%) did not differ significantly between groups. The calcium-supplemented group had significantly higher fecal calcium density ($P < 0.001$), higher serum calcium concentrations ($P < 0.009$), lower serum PTH concentrations ($P < 0.001$), and higher urinary calcium excretion ($P < 0.006$) (**Figure 2**) than did the placebo group. PTH concentrations in the placebo group reached their peak by the onset of menarche and declined thereafter, subsequent to changes in serum calcium. Fecal calcium density and urinary calcium were

TABLE 2
Dietary and total calcium intake by age of subjects in calcium-supplemented and placebo groups[1]

Age (y)	Supplemented group		Placebo group dietary (total) calcium intake
	Total calcium intake	Dietary calcium intake	
	mg/d		*mg/d*
11	855 ± 288 (134)	855 ± 288	819 ± 302 (157)
11.5	1589 ± 373 (114)	859 ± 305	826 ± 294 (133)
12	1569 ± 352 (103)	791 ± 284	772 ± 270 (126)
12.5	1577 ± 330 (114)	809 ± 256	841 ± 304 (130)
13	1584 ± 390 (111)	828 ± 287	825 ± 325 (131)
13.5	1607 ± 430 (97)	890 ± 348	855 ± 351 (123)
14	1560 ± 455 (99)	861 ± 296	861 ± 327 (120)
14.5	1533 ± 399 (92)	853 ± 311	822 ± 344 (116)
15	1544 ± 457 (96)	869 ± 379	792 ± 395 (114)
15.5	1504 ± 500 (79)	908 ± 408	763 ± 373 (100)
16	1336 ± 539 (79)	790 ± 331	805 ± 417 (100)
16.5	1441 ± 528 (64)	844 ± 353	768 ± 346 (91)
17	1396 ± 609 (69)	839 ± 391	805 ± 376 (87)
17.5	1427 ± 588 (60)	873 ± 382	866 ± 416 (79)
18	1296 ± 567 (79)	824 ± 351	830 ± 377 (100)

[1] All values are $\bar{x}$ ± SD; *n* in parentheses. Two-sample *t* test for the difference between the total calcium intake in the placebo and calcium-supplemented groups found no difference between the groups at average age 11 y (baseline: $P < 0.3$) and a highly significant difference between the groups at each age level above age 11 y ($P < 0.0001$). The difference between the dietary calcium intakes in the 2 groups was not significant at any age level.

lower in both groups during the premenarcheal period than after menarche. The trend was not clinically significant, but the fact that the pattern changes at menarche is clinically significant.

Bone turnover markers

For serial measurements of the markers of bone formation (ie, serum alkaline phosphatase and osteocalcin) and bone resorption (urinary NTX), the YSM was the best descriptor of the events associated with puberty. The markers all reached their peak near the onset of menarche and declined thereafter. There were no significant differences in serum total alkaline phosphatase ($P = 0.30$), serum osteocalcin (ng/mL) ($P = 0.69$; **Figure 3**), and urinary NTX excretion (nmol · L bone collagen equivalent/d; $P = 0.31$; Figure 3) between supplemented and placebo groups.

Stature, bone width, and bone mineral area

After 4 y of intervention there was no significant difference in gains in stature, TA, and bone mineral areas between the calcium-supplemented and placebo groups (**Table 3**). Nor were differences in gains in stature, metacarpal TA, and bone mineral areas confirmed after 7 y of intervention (**Table 4**). In addition, a repeated-measure MANOVA for height, metacarpal TA, and bone mineral areas, in which baseline values were used as covariates, showed no significant group × time interactions or group main effects.

Bone mineral density and metacarpal radiogrammetry

Because the original clinical trial was set up for a 4-y duration, we first present analyses of the primary outcomes for this cohort, to dispel the possible idea that the extension of the trial may have been conducted to achieve significant results. The general MANOVA on primary outcome variables (visits 2–9) for the 4-y cohort, in which baseline values were used as covariates, and the general MANOVA for the gain from baseline (supplemented group: $n = 100$, placebo group: $n = 120$) indicated highly significant treatment effects on TBBMD (covariate analysis: $P < 0.0003$; gain: $P < 0.0006$). Similar analyses for proximal (supplemented group: $n = 91$, placebo group: $n = 108$) and distal (supplemented group: $n = 93$, placebo group: $n = 111$) radius BMD, in which baseline values were used as covariates, found significant effect for distal radius BMD only ($P < 0.0026$). The repeated-measures MANOVA for these 3 variables (visits 1–9) and for their gain from baseline (visits 2–9), in which baseline values were used as covariates, found that, for the BMD measurements, group × visits interactions are all highly significant ($P < 0.028$), but, for the gains from the baseline, none of these interactions are significant ($P > 0.572$). The repeated-measures MANOVA for metacarpal CA and CA:TA (supplemented group: $n = 101$, placebo group: $n = 118$), in which baseline values were used as covariates, found significant interaction effects for CA:TA but an insignificant interaction effect for CA (ratio of CA to TA: $P < 0.02$; CA: $P > 0.09$). The ANOVA for gain from baseline, in which baseline values were used as covariates, showed significant differences between the groups for CA:TA ($P < 0.024$). Table 3 shows the data for these variables at baseline and at the year 4 endpoint and their gains from the baseline, along with the follow-up univariate analyses for BMD at the 3 sites and for metacarpal CA and CA:TA. The follow-up univariate analyses of these data at the year 4 endpoint show that TBBMD, proximal and distal radius BMD, metacarpal CA:TA, and the gains in TBBMD and metacarpal CA:TA, when baseline measurements were used as covariates, were significantly higher in the calcium-supplemented group than in the placebo group.

For the 7-y cohort, the general MANOVA for comparing the mean responses on primary outcome variables (visits 2–15), in which their baseline values were used as covariates, found a highly significant treatment effect on TBBMD (supplemented group: $n = 60$; placebo group: $n = 67$; $P < 0.0004$), proximal radius BMD (supplemented group: $n = 56$; placebo group: $n = 63$; $P < 0.024$), and distal radius BMD (supplemented group: $n = 57$; placebo group: $n = 64$; $P < 0.028$). The repeated-measures

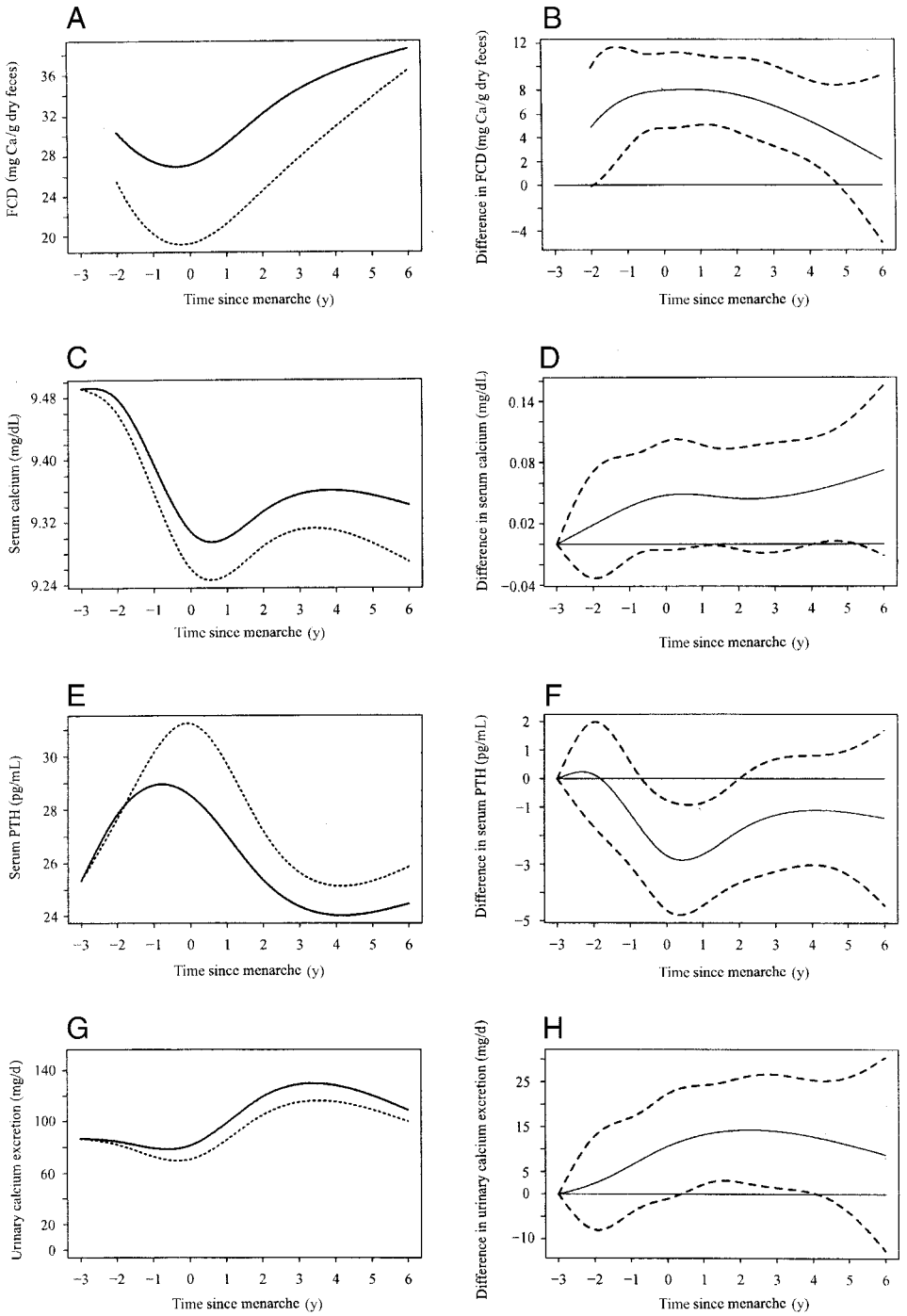

FIGURE 2. Longitudinal patterns based on the linear mixed-effect (LME) model for the supplemented (—) and placebo (- - -) groups for biochemistry data with years since menarche and the corresponding 95% CIs for their difference. A and B: supplemented group, $n = 106$; placebo group, $n = 125$; C and D: supplemented group, $n = 116$; placebo group, $n = 137$; E and F: supplemented group, $n = 115$; placebo group, $n = 137$; G and H: supplemented group, $n = 115$; placebo group, $n = 137$. The CI provides the estimated range of values for years since menarche during which the 2 groups differ significantly; when the CI contains zero, the difference at that time point is not significant. FCD, fecal calcium density; PTH, parathyroid hormone.

MANOVA (visits 1–15), in which baseline values were used as covariates, found highly significant treatment × visits interaction for all 3 variables ($P < 0.0006$). The repeated-measures MANOVA of the gains from the baseline for this cohort, in which baseline measurements were used as covariates, found highly significant treatment × visit interactions in TBBMD ($P < 0.0097$) and proximal radius BMD ($P < 0.001$). A subanalysis of gain from the baseline by using general MANOVA on the follow-up data from the first 4 y only (visits 2–9) found that the supplemented and placebo groups were significantly different (TBBMD: $P < 0.0004$; proximal radius BMD: $P < 0.004$). However, the general MANOVA for the gains from baseline for the same cohort during the 3-y study extension (visits 10–15) did not find a difference between the groups (TBBMD: $P < 0.220$;

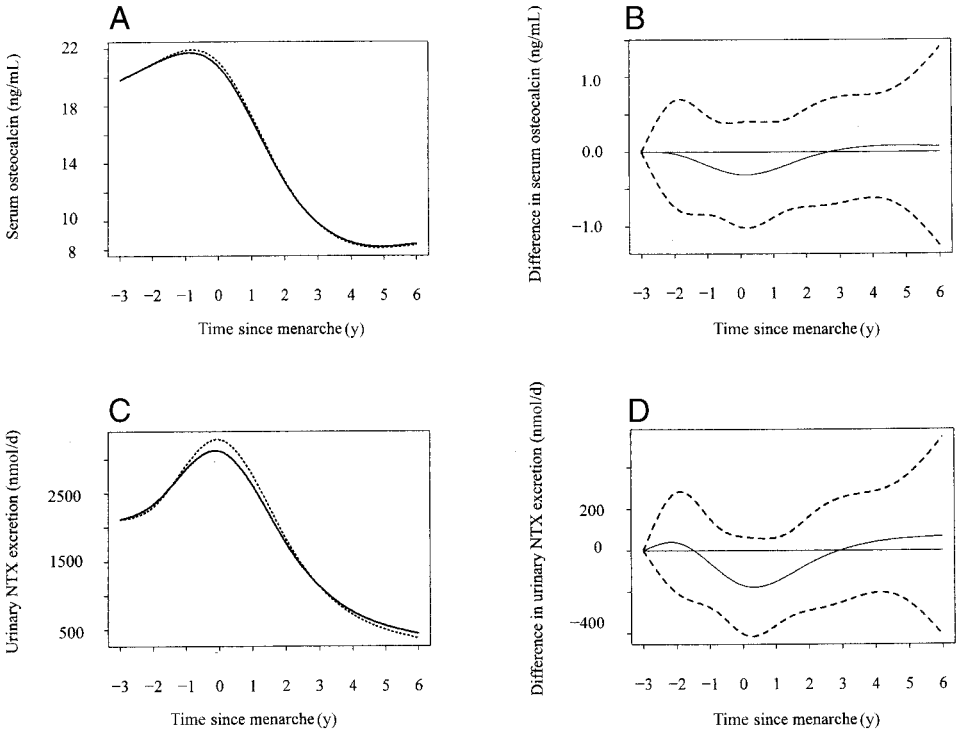

FIGURE 3. Longitudinal patterns based on the linear mixed-effect (LME) model for the supplemented (—) and placebo (- - -) groups for bone turnover biomarkers with years since menarche and the corresponding 95% CIs for their difference. A and B: supplemented group, $n = 116$; placebo group, $n = 137$; C and D: supplemented group, $n = 115$; placebo group, $n = 137$. The differences between the groups were not significant for osteocalcin and urinary *N*-telopeptide. The CI provides the estimated range of values for years since menarche during which the 2 groups differ significantly; when the CI contains zero, the difference at that time point is not significant.

proximal radius BMD: $P < 0.346$). The repeated-measures MANOVA for metacarpal CA and CA:TA, in which baseline values were used as covariates (visits 1, 9, 15; supplemented group: $n = 76$; placebo group: $n = 96$), found that treatment × visit interactions were significant for these variables (CA: $P < 0.024$; ratio of CA to TA: $P < 0.0034$). However, for the gains from baseline, when baseline measurements were used as covariates, the interaction was significant for CA:TA ($P < 0.0000$) but not for CA ($P > 0.11$). Table 4 provides data for these variables at baseline, at the year 4 and year 7 endpoints, and their gains from the baseline, along with the results of follow-up univariate analyses for BMD at the metacarpal CA and CA:TA,

TABLE 3
Stature and bone outcome measures in the 4-y cohort of subjects in the supplemented and placebo groups at baseline and year 4[1]

	Baseline		Year 4		Gain	
	Supplemented group	Placebo group	Supplemented group	Placebo group	Supplemented group	Placebo group
Height (cm)	145.1 ± 7.4	145.1 ± 7.0	163.2 ± 5.6	163.5 ± 6.1	18.0 ± 5.2	18.4 ± 4.1
Total-body BMD (g/cm^2)	0.893 ± 0.057	0.891 ± 0.059	1.108 ± 0.066	1.096 ± 0.072[2]	0.215 ± 0.037	0.204 ± 0.035[3]
Total-body BMA (cm^2)	1466 ± 170	1457 ± 180	2040 ± 167	2036 ± 212	575 ± 127	579 ± 132
Proximal radius BMD (g/cm^2)	0.501 ± 0.047	0.498 ± 0.048	0.654 ± 0.048	0.643 ± 0.053[2]	0.153 ± 0.029	0.145 ± 0.031
Proximal radius BMA (cm^2)	2.077 ± 0.192	2.084 ± 0.192	2.247 ± 0.195	2.254 ± 0.194	0.167 ± 0.088	0.171 ± 0.079
Distal radius BMD (g/cm^2)	0.280 ± 0.039	0.276 ± 0.040	0.386 ± 0.054	0.368 ± 0.057[2]	0.106 ± 0.047	0.092 ± 0.046
Distal radius BMA (cm^2)	2.248 ± 0.261	2.228 ± 0.231	2.655 ± 0.237	2.668 ± 0.259	0.407 ± 0.171	0.441 ± 0.180
Calcium (cm^2)	0.136 ± 0.020	0.133 ± 0.017	0.186 ± 0.023	0.179 ± 0.020	0.049 ± 0.013	0.046 ± 0.012
TA (cm^2)	0.392 ± 0.052	0.393 ± 0.051	0.436 ± 0.052	0.436 ± 0.052	0.043 ± 0.018	0.044 ± 0.017
CA:TA	0.348 ± 0.039	0.341 ± 0.037	0.427 ± 0.042	0.414 ± 0.047[2]	0.079 ± 0.021	0.072 ± 0.021[3]

[1] All values are $\bar{x}$ ± SD. BMD, bone mineral density; BMA, bone mineral area; CA, metacarpal cortical area; TA, metacarpal total area. Univariate two-sample *t* test for gain and analysis of covariance for the placebo ($n = 121$) and supplemented ($n = 101$) groups, with baseline values as covariate, at the 4-y endpoint, whenever the repeated-measures multivariate analysis of variance (MANOVA) indicated a significant interaction effect or the general MANOVA indicated significant differences in the mean responses. Because the MANOVAs ignore a case with even one missing observation, the number of subjects reported in the text for some of the MANOVAs was substantially less than the number of subjects reported in the table, which includes all subjects who were measured at the endpoint.

[2,3] Significantly different from baseline: [2]$P < 0.01$, [3]$P < 0.05$.

TABLE 4
Stature and bone outcome measures of the 7-y cohort of subjects in the supplemented and placebo groups at baseline and at years 4 and 7[1]

	Baseline		Year 4		Gain at 4 y		Year 7		Gain at 7 y	
	Supplemented group	Placebo group	Supplemented group	Placebo group	Supplemented group	Placebo group	Supplemented group	Placebo group	Supplemented group	Placebo group
Height (cm)	145.7 ± 7.3	144.7 ± 7.0	163.4 ± 5.5	163.2 ± 6.1	17.7 ± 4.8	18.5 ± 4.0	165.2 ± 5.6	164.9 ± 6.1	19.5 ± 5.8	20.2 ± 5.0
Total-body BMD (g/cm^2)	0.892 ± 0.055	0.888 ± 0.057	1.105 ± 0.067	1.094 ± 0.070[2]	0.213 ± 0.037	0.206 ± 0.036	1.160 ± 0.071	1.152 ± 0.066	0.268 ± 0.049	0.263 ± 0.044
Total-body BMA (cm^2)	1475 ± 171	1444 ± 178	2043 ± 172	2022 ± 205	568 ± 122	579 ± 124	2161 ± 198	2154 ± 225	686 ± 174	710 ± 164
Proximal radius BMD (g/cm^2)	0.502 ± 0.046	0.496 ± 0.049	0.654 ± 0.049	0.641 ± 0.053[3]	0.152 ± 0.029	0.146 ± 0.031	0.664 ± 0.049	0.652 ± 0.049[2]	0.162 ± 0.038	0.156 ± 0.036
Proximal radius BMA (cm^2)	2.071 ± 0.197	2.079 ± 0.197	2.240 ± 0.204	2.239 ± 0.195	0.166 ± 0.090	0.169 ± 0.078	2.326 ± 0.207	2.337 ± 0.213	0.255 ± 0.099	0.262 ± 0.089
Distal radius BMD (g/cm^2)	0.279 ± 0.039	0.273 ± 0.040	0.387 ± 0.058	0.366 ± 0.058[3]	0.108 ± 0.050	0.093 ± 0.048	0.450 ± 0.053	0.438 ± 0.050	0.171 ± 0.047	0.165 ± 0.040
Distal radius BMA (cm^2)	2.252 ± 0.261	2.230 ± 0.231	2.646 ± 0.240	2.656 ± 0.265	0.394 ± 0.161	0.430 ± 0.187	2.585 ± 0.244	2.594 ± 0.250	0.333 ± 0.199	0.364 ± 0.169
CA (cm^2)	0.137 ± 0.020	0.132 ± 0.017	0.186 ± 0.024	0.178 ± 0.021[3]	0.049 ± 0.013	0.046 ± 0.012[2]	0.196 ± 0.024	0.186 ± 0.020[3]	0.059 ± 0.015	0.054 ± 0.014[2]
TA (cm^2)	0.392 ± 0.053	0.392 ± 0.049	0.436 ± 0.053	0.434 ± 0.049	0.042 ± 0.018	0.044 ± 0.018	0.443 ± 0.052	0.440 ± 0.047	0.049 ± 0.020	0.050 ± 0.019
CA:TA	0.349 ± 0.036	0.339 ± 0.035	0.429 ± 0.040	0.412 ± 0.047[3]	0.079 ± 0.020	0.072 ± 0.022[2]	0.445 ± 0.043	0.425 ± 0.048[3]	0.095 ± 0.025	0.085 ± 0.024[2]

[1] All values are $\bar{x}$ ± SD. BMD, bone mineral density; BMA, bone mineral area; CA, metacarpal cortical area; TA, metacarpal total area. Univariate two-sample *t* test for gain and analysis of covariance for the placebo ($n = 98$) and supplemented ($n = 79$) groups, with baseline values as covariate, at the 4-y and 7-y endpoints, whenever the repeated-measures multivariate analysis of variance (MANOVA) indicated a significant interaction effect or the general MANOVA indicated significant differences in the mean responses. Because the MANOVAs ignore a case with even one missing observation, the number of subjects reported in the text for some of the MANOVAs was substantially less than the number of subjects reported in the table, which includes all subjects who were measured at the endpoint(s).

[2,3] Significantly different from baseline: [2]$P < 0.05$, [3]$P < 0.01$.

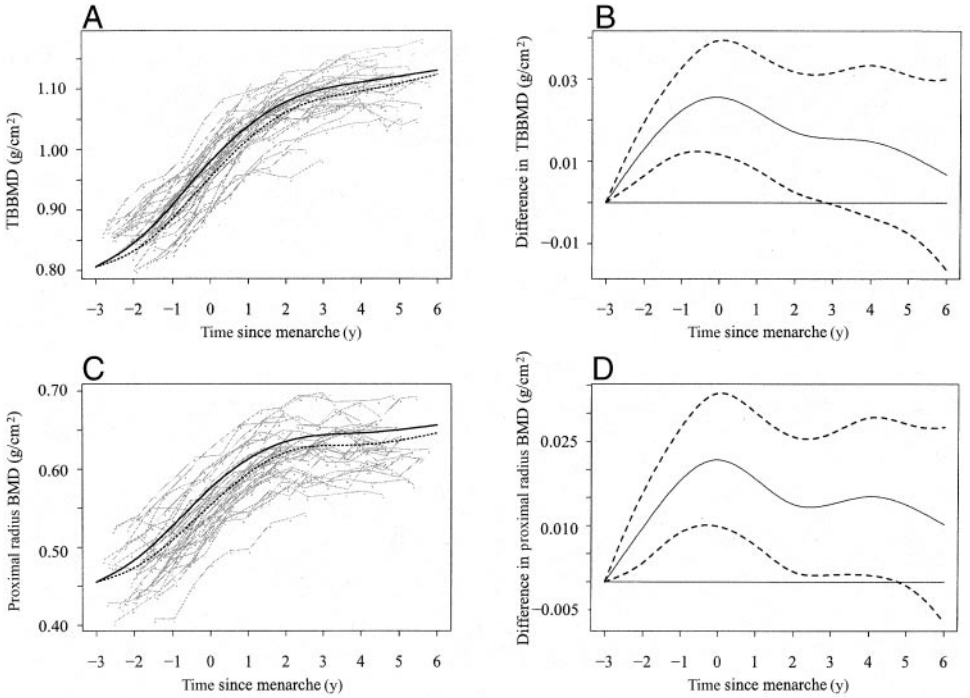

FIGURE 4. Longitudinal patterns based on the linear mixed-effect (LME) model for the supplemented (—) and placebo (- - -) groups for bone mineral density (BMD) with years since menarche and the corresponding 95% CIs for their difference. A and B: supplemented group, $n = 115$; placebo group, $n = 137$; C and D: supplemented group, $n = 115$; placebo group, $n = 137$. Gray background in panels A and C shows patterns of 15 randomly selected persons in each group. The CI provides the estimated range of values for years since menarche during which the 2 groups differ significantly; when the CI contains zero, the difference at that time point is not significant.

adjusted for the baseline values. The differences between the supplemented and placebo groups in TBBMD and distal BMD, which were significant at the year 4 endpoint, were no longer significant at the year 7 endpoint. In addition, the metacarpal CA and CA:TA and their gains from baseline were significantly higher in the supplemented group than in the placebo group at both these endpoints (Table 4). Collectively, these analyses clearly indicated a need for further examination of the longitudinal behavior of bone mass accretion in the supplemented and placebo groups.

The longitudinal analysis based on the LME model revealed a significant influence of calcium supplementation on TBBMD ($P < 0.001$) and proximal radius BMD ($P < 0.001$) (**Figure 4**). The calcium-supplemented group showed a faster rate of bone mass accretion from the beginning of the study; the maximum difference between the BMD of the 2 groups at both of these sites occurred during the interval from −1 YSM to +1 YSM. After +1 YSM, the differences between the groups started to diminish, and they became insignificant after +3 and +5 YSM for TBBMD and proximal radius BMD, respectively (Figure 4).

A post hoc stratification of subjects based on the compliance-adjusted average cumulative total calcium intake, irrespective of assigned group, showed significant influence of calcium on the proximal radius BMD pattern ($P < 0.0001$) (**Figure 5**). The high-calcium-intake subgroup (average intake: 1353 ± 342 mg/d; $n = 180$) has persistently higher BMD of the proximal radius

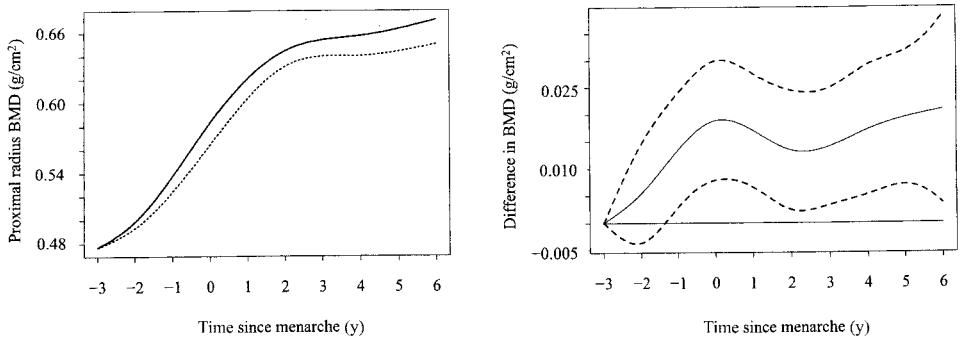

FIGURE 5. Longitudinal patterns based on the linear mixed-effect (LME) model for the post hoc strata with high (—) and low (- - -) calcium intakes, irrespective of assigned group, for the proximal radius bone mineral density (BMD) with years since menarche and the corresponding 95% CIs for their difference: high-calcium-intake subgroup, $n = 180$ (average cumulative calcium intake: 1353 ± 342 mg/d); low-calcium-intake subgroup, $n = 89$ (average cumulative calcium intake: 668 ± 118 mg/d). The CI provides the estimated range of values for years since menarche during which the 2 groups differ significantly; when the CI contains zero, the difference at that time point is not significant.

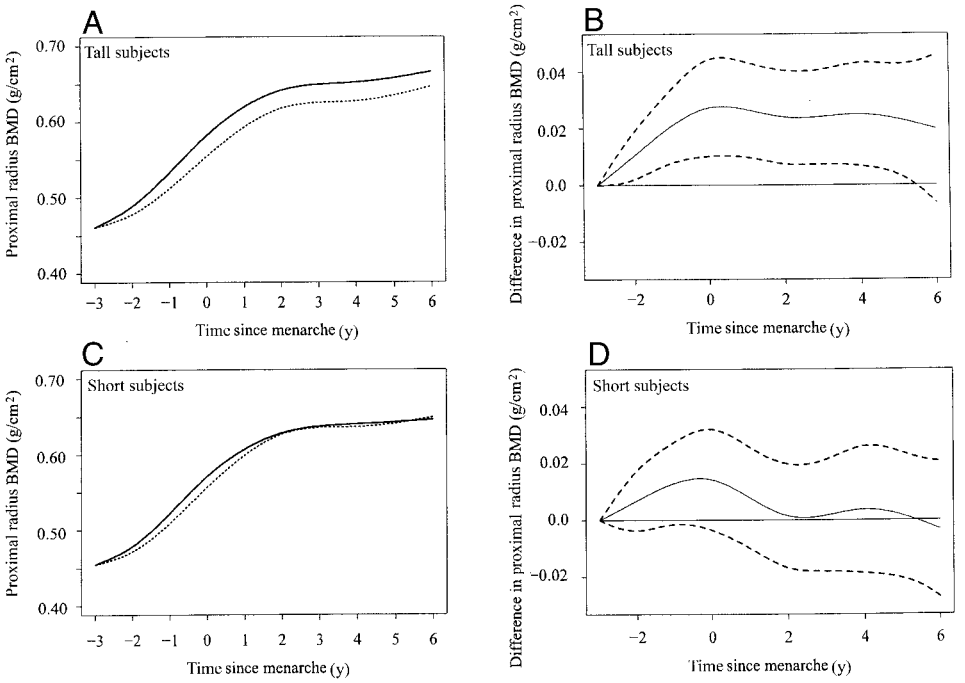

FIGURE 6. Longitudinal patterns based on the linear mixed-effect (LME) model for the supplemented (—) and placebo (- - -) groups for bone mineral density (BMD) of the proximal radius with years since menarche and the corresponding 95% CIs for their difference, with the post hoc substratification based on the final stature (height above and below the median). A and B: supplemented group, $n = 50$; placebo group, $n = 62$; C and D: supplemented group, $n = 53$; placebo group, $n = 60$. The CI provides the estimated range of values for years since menarche during which the 2 groups differ significantly; when the CI contains zero, the difference at that time point is not significant. The results show a significant interaction between body size (stature) and treatment.

from puberty to young adulthood than does the low-calcium-intake subgroup (average intake: 668 ± 118 mg/d; $n = 89$).

Skeletal size and calcium requirement

According to the two-factor (ie, pill or placebo and short or tall) ANCOVA of the proximal radius BMD, the overall F test indicated a significant effect ($P < 0.04$) at the year 4 endpoint and an insignificant effect ($P = 0.23$) at the year 7 endpoint. At year 4, the interaction between these 2 factors showed that, for taller subjects, the contrast between the placebo and calcium-supplemented groups was significant ($P < 0.006$), whereas, for shorter subjects, the test for the contrast did not reveal any significant effect ($P = 0.826$). Consequently, we performed separate LME analysis for the tall and short subgroups, which found a significant influence of calcium supplementation on bone mineral accretion at proximal radius in tall persons. The maximum difference between the BMD of the 2 groups was during the interval ±1 YSM. After +5 YSM, the differences between the groups started to diminish and became insignificant after +5.5 YSM (**Figure 6**). Unlike the tall subgroup, the short subgroup did not show an effect of calcium supplementation on the proximal radius BMD (Figure 6).

The F test in the two-factor (ie, pill or placebo and larger or smaller bones) ANCOVA of the metacarpal CA found significant overall effects at both the year 4 ($P < 0.006$) and the year 7 ($P < 0.005$) endpoints. The interaction between these 2 factors showed that, among subjects with larger metacarpals, the contrasts between the placebo and calcium-supplemented groups were highly significant at both the year 4 ($P < 0.02$) and year 7 ($P < 0.002$) endpoints; however, the effect was not present among subjects with smaller metacarpals at either year 4 ($P = 0.79$) or year 7 ($P = 0.70$). This implies that a calcium intake of ≈830 mg/d was adequate for persons with smaller metacarpals but insufficient for those genetically predetermined to develop larger bones.

Bone fracture

Twenty girls from the placebo group and 9 girls from the calcium-supplemented group reported having a bone fracture because of a moderate trauma during the trial. There were 3 forearm fractures in the supplemented group (33%) and 11 in the placebo group (48%). The average timing of the fracture was +1.2 ± 0.4 YSM, which coincides with the bone-modeling phase in skeletal development and overlaps with the timing of the maximal effect of calcium supplementation on BMD among young females in this study (Figure 4).

DISCUSSION

This study documents that calcium supplementation (≈670 mg/d) beyond a habitual dietary calcium intake of ≈830 mg/d (increasing total calcium intake up to ≈1500 mg/d) positively influences bone mass acquisition throughout the bone-modeling phase of the pubertal growth spurt. This effect diminishes during the skeletal consolidation of late adolescence that is due to the catch-up phenomenon in bone mineral accretion. At the beginning of young adulthood, the positive effect of calcium supplementation was evident at all skeletal regions of interest; however, the only differences that remained significant were those at the metacarpals and at the proximal radius among the subgroup with high calcium intake and in tall persons.

The explanation for the catch-up phenomenon lies within the skeletal physiology of growth, which dictates calcium requirement (4, 12). Calcium requirement is the highest during the pubertal growth spurt, when most of the retained calcium contributes to skeletal build-up to accommodate longitudinal bone growth and periosteal bone expansion (4, 13). During this interval, ≈37% of the entire adult skeletal mass is accumulated (13). Therefore, inadequate calcium intake during this period compromises the bone mineral accretion rate (3). During the pubertal growth spurt, there was an obvious discrepancy between optimal and actual calcium intakes among the placebo group in this study, which was possibly reflected in a higher fracture rate, even though that was not a research outcome variable. However, when bone modeling slows down (by epiphyseal closure that results from increased estradiol secretion after menarche), the demand for calcium declines, and the calcium intake threshold decreases from 1480 to 957 mg/d (12). The habitual calcium intake in the young women in the current study is very close to this reduced requirement and is sufficient to accommodate bone consolidation, which allows BMD to catch up slowly.

These conclusions are supported by the blood, stool, and urine chemistry measurements. During bone modeling of the pubertal growth spurt, the PTH concentrations in the placebo group reached a maximum and declined thereafter. The increase in PTH could increase intracortical remodeling and porosity as indicated previously (36) and is reflected in a slightly increased urinary NTX excretion. The pubertal growth spurt is the time when serum calcitriol concentration is at its maximum and is increasing calcium absorption to accommodate skeletal needs (37), which results in a lower fecal calcium excretion (4), and presumably in a lower fecal calcium density. Calcium absorption decreases during bone consolidation in late adolescence (4), and fecal calcium density increases concomitantly. Calcium supplementation during the pubertal growth spurt partially blunts PTH secretion, which suggests that calcium intake is adequate, and bone resorption decreases concomitantly, as previously documented by calcium kinetics (38). The changes in serum PTH reflect the fluctuations in serum calcium concentration during growth. Serum calcium is lower during the bone-modeling phase of the pubertal growth spurt than it is either before that phase or later, during the bone-consolidation phase. Urinary calcium follows those changes closely (4). Urinary calcium excretion is lower during the bone-modeling phase than during skeletal consolidation. The difference in urinary calcium excretion between the 2 groups was much smaller during the pubertal growth spurt (7%) than it was in late adolescence (18%), which implies that urinary calcium is less dependent on intake during bone modeling, as documented by balance studies (3, 4). Calcium supplementation increased urinary calcium more during bone consolidation, despite a drop in calcium intake (by 10%), which suggests that the calcium supply is adequate.

The catch-up phenomenon in bone mass acquisition suggests the existence of a reversible mineral deficit that is acquired during the pubertal growth spurt (36) and that can be repaired during bone consolidation. The catch-up phenomenon was apparent, in particular at the total body. However, at the metacarpals and at the proximal forearm of persons accustomed to different calcium intakes, the difference created during bone modeling was maintained into young adulthood, which implies incomplete catch-up and a lower peak bone mass. The lower the habitual calcium intake in the population, the higher the possibility of a low peak bone mass at skeletal maturity. The results of ecologic studies (1, 39) conducted among populations accustomed to very low lifetime calcium intakes point in this direction. Metacarpal radiogrammetric measurements (ie, CA and CA:TA) were lower in women from a low-calcium-intake area of Croatia, who were accustomed to calcium intakes of ≈400 mg/d over a lifetime and who had a concomitantly high rate of hip fracture (1). Similar findings with regard to peak bone mass were obtained in animal experimentations by manipulating calcium in the diet early in life (40, 41).

In addition to BMD, the current study evaluated the effect of calcium supplementation on longitudinal (height) and periosteal (width) bone expansion and on bone mineral area. The results show that calcium supplementation in addition to the habitual calcium intake of ≈830 mg/d had no effect on bone geometry measurements.

To ascertain whether BMD is related to calcium supplementation among persons of different body frames, its interactions with final height and periosteal expansion (TA) were examined (33, 34). Among subjects destined to be taller or to have larger bones, those with higher calcium intakes had significantly higher BMD than did those with lower calcium intakes. These results strongly support the notion that dietary calcium requirement for skeletal development is size dependent.

All clinical trials with calcium or dairy product supplementation in children and adolescents that have been completed to date (3, 5–10, 42–44) showed a positive effect of intervention on bone mass, but they were all too short (1–3 y) to address the question of whether it is the adaptation of bone tissue to nutritional challenge that leads to peak bone mass. The increase in bone mass observed in those short-term studies could be explained to a large extent by the phenomenon of the bone-remodeling transient (45). In some of the studies reported earlier, the difference in bone mass between the groups diminished after calcium intervention was discontinued (46, 47), which indicated that the bone accretion gained in the first transient was lost as a result of the second (45). In other studies, the effects of intervention were maintained 1–3 y after discontinuation of treatment (44, 48, 49); this result may be specific to the calcium source, to the amount of habitual dietary calcium intake, or both.

In the current study, however, calcium supplementation continued without interruption for 7 y, which allowed for the adaptation in bone behavior during the bone-modeling and bone-consolidation phases in skeletal development and extended long after the first bone-remodeling transient effect ended (≈12 mo after the beginning of intervention). According to calcium balance studies (4), the calcium requirement of the participants during the 7-y follow-up was changing as well. The effect of the second transient, following the discontinuation of calcium supplementation after 7 y, therefore is not expected because dietary calcium requirements have been reduced to an amount closer to habitual dietary calcium intake. Thus, if we assume that these women will maintain their dietary habits, it is unlikely that the differences in bone mass measurements observed at these skeletal regions of interest at the beginning of young adulthood will disappear by the cessation of intervention. A follow-up study may be necessary for confirmation.

Some studies indicate that calcium intake may play a role in the prevention of fractures due to bone fragility during growth (50, 51). The fracture rate was not a primary research outcome, but the reported fracture incidence in our study points in this direction,

although the sample size may be too small for adequate interpretation. Because the peak incidence of fractures due to bone fragility coincides with the pubertal growth spurt (52, 53), our results indicate that calcium intakes at threshold level may reduce the risk of fracture during this stage of skeletal development, irrespective of the catch-up phenomenon in bone mass acquisition that occurs thereafter. The possibility that calcium intakes may reduce the risk of fracture is particularly important given the large number of childhood forearm fractures and the rising incidence over the last 30 y (54). A long-term intervention study of calcium supplementation in children that evaluates the forearm fracture as the main research outcome should be conducted to resolve this issue.

It is of great interest to pediatricians to tailor the nutritional recommendations for adolescents to their individual calcium needs, which are maximal at the pubertal growth spurt. However, such specification may be difficult to implement in practice because a person's rates of bone growth and skeletal maturity cannot be predicted precisely. The results of the current study also have implications for dietary calcium intake standards for children and adolescents worldwide; the standards for one ethnic group might not be suitable for another. Each country should develop its own standards that are specific to the people living in the region. Factors such as ethnicity, dietary habits (including salt intake), sunlight exposure, and activity level all play a role, but stature and body frame must also be considered.

In summary, this study documents that calcium supplementation in excess of a habitual calcium intake of ≈830 mg/d affects BMD during the pubertal growth spurt, but there is a diminishing effect thereafter that is due to the catch-up phenomenon in bone mineral accretion. By young adulthood, significant effects of calcium supplementation were present at metacarpals and at the proximal forearm in subjects who had better calcium compliance and in subjects who developed larger body frames. These results imply that standards for dietary calcium intake in adolescence should be based on growth rate and body and bone size development. The results of this study may be important for the prevention of bone fragility fractures during growth, as well as for the primary prevention of osteoporosis.

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VM, TNH, and PKG were involved in the study design and study conduction; NEBS, JDL, JZI, MS, LAN, SLM, and EJH were involved in various phases of data collection; PKG and BL performed the statistical analysis; TNH was involved in designing and implementing quality control for the DXA measurements; NEBS, JZI, and JDL were involved in database development and maintenance; VM, PKG, BL, AC, JDL, and JZI were involved in data analysis; VM and PKG had a major role in writing the manuscript; and all authors contributed to the writing of the manuscript. All authors have declared that no conflicts of interest exist.

REFERENCES

1. Matkovic V, Kostial K, Simonovic I, Buzina R, Brodarec A, Nordin BEC. Bone status and fracture rates in two regions of Yugoslavia. Am J Clin Nutr 1979;32:540–9.
2. Heaney RP, Abrams S, Dawson-Hughes B, et al. Peak bone mass. Osteoporosis Int 2000;11:985–1009.
3. Matkovic V, Fontana D, Tominac C, Goel P, Chesnut CH. Factors which influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. Am J Clin Nutr 1990; 52:878–88.
4. Matkovic V. Calcium metabolism and calcium requirements during skeletal modeling and consolidation of bone mass. Am J Clin Nutr 1991;54(suppl):245S–60S.
5. Johnston CC Jr, Miller JZ, Slemenda CW, et al. Calcium supplementation and increases in bone mineral density in children. N Engl J Med 1992;327:82–7.
6. Lloyd T, Andon MB, Rollings N, et al. Calcium supplementation and bone mineral density in adolescent girls. JAMA 1993;270:841–4.
7. Lee WTK, Leun SSF, Wang SF, et al. Double-blind, controlled calcium supplementation and bone mineral accretion in children accustomed to a low-calcium diet. Am J Clin Nutr 1994;60:744–50.
8. Bonjour JP, Carrie AL, Ferrarri S, Clavien H, Slosman D, Theintz G, Rizzoli R. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled, trial. J Clin Invest 1997;99:1287–94.
9. Nowson CA, Green RM, Hopper JL, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. Osteoporosis Int 1997;7:219–25.
10. Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EME. Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. Am J Clin Nutr 2000; 71:544–9.
11. Ilich JZ, Skugor M, Hangartner T, Baoshe A, Matkovic V. Relation of nutrition, body composition, and physical activity to skeletal development: a cross-sectional study in preadolescent females. J Am Coll Nutr 1998;17:136–47.
12. Matkovic V, Heaney RP. Calcium balance during human growth: evidence for threshold behavior. Am J Clin Nutr 1992;55:992–6.
13. Matkovic V, Jelic T, Wardlaw GM, et al. Timing of peak bone mass in caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest 1994;93:799–808.
14. Ma D, Jones G. The association between bone mineral density, metacarpal morphometry, and upper limb fractures in children: a population-based case-control study. J Clin Endocrinol Metab 2003;88:1486–91.
15. Matkovic V, Ilich JZ, Skugor M, et al. Leptin is inversely related to age at menarche in human females. J Clin Endocrinol Metab 1997;82:3239–45.
16. Ilich JZ, Hangartner TA, Skugor M, Roche AF, Goel P, Matkovic V. Skeletal age as a determinant of bone mass in young females. Skeletal Radiol 1996;25:431–9.
17. Jorgensen JT, Andersen PB, Rosholm A, Bjarnason NH. Digital X-ray radiogrammetry: a new appendicular bone densitometric method with high precision. Clin Physiol 2000;20:330–5.
18. Landoll JD, Badenhop-Stevens NE, May R, Hangartner T, Roehrig JL, Matkovic V. Comparison of the X-Posure System for radiogrammetry of the metacarpals with standard techniques and DXA. J Bone Miner Res 2001;16:S457 (abstr).
19. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. Am J Clin Nutr 1983;37:461–5.
20. Heaney RP. Fecal calcium density: a measure of calcium compliance. J Bone Miner Res 1991;6:469–71.
21. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an ^{125}I-labeled tracer. Clin Chem 1993;39:529–33.
22. Everitt BS, Pickles A. Statistical aspects of the design and analysis of clinical trials. London: Imperial College Press, 1999.
23. Sommer A, Zeger SL. On estimating efficacy from clinical trials. Stat Med 1991;10:45–52.

24. Laird NM, Ware JH. Random-effects model for longitudinal data. Biometrics 1982;38:963–74.
25. Pinheiro JC, Bates D. Mixed-effects models in S and S-PLUS. New York: Springer, 2000.
26. Weaver CM, Martin BR, Plawecki KL, et al. Differences in calcium metabolism between adolescent and adult females. Am J Clin Nutr 1995;61:577–81.
27. Abrams SA, O'Brien KO, Stuff JE. Changes in calcium kinetics associated with menarche. J Clin Endocrinol Metab 1996;81:2017–20.
28. Matkovic V. Bone turnover and skeletal development revisited. J Clin Endocrinol Metab 1996;81:2013–6.
29. Wang YX, Taylor JMG. Inference for smooth curves in longitudinal data with application to AIDS clinical trial. Stat Med 1995;14:1205–18.
30. The European Collaborative study. Height, weight, and growth in children born to mothers with HIV-1 infection in Europe. Pediatrics 2003; 111:e52–60.
31. Lambert PC, Abrams KR, Jones DR, Halligan AWF, Shenan A. Analysis of ambulatory blood pressure monitor data using a hierarchical model incorporating restricted cubic splines and heterogenous within-subject variances. Stat Med 2001;20:3789–805.
32. Bachrach L, Hastie T, Wang M-C, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black and caucasian youth: a longitudinal study. J Clin Endocrinol Metab 1999;84:4702–12.
33. Matkovic V, Ilich JZ. Calcium requirements during growth. Are the current standards adequate? Nutr Rev 1993;51:171–80.
34. Matkovic V, Badenhop NE, Ilich JZ. Trace element and mineral nutrition in healthy people: adolescents. In: Bogden JD, Klevay LM, eds. Clinical nutrition of the essential trace elements and minerals—the guide for health professionals. Totowa, NJ: Humana Press, 2000:153–82.
35. Everitt BS, Rabe-Hasketh S. Analyzing medical data using S-plus. New York: Springer, 2001.
36. Parfitt AM. The two faces of growth: benefits and risks to bone integrity. Osteoporosis Int 1994;4:382–98.
37. Ilich JZ, Badenhop NE, Jelic T, Clairmont AC, Nagode LA, Matkovic V. Calcitriol and bone mass accumulation in females during puberty. Calcif Tissue Int 1997;61:104–9.
38. Wastney ME, Martin BR, Peacock M, et al. Changes in calcium kinetics in adolescent girls induced by high calcium intake. J Clin Endocrinol Metab 2000;85:4470–5.
39. Hu JF, Zhao XH, Jia JB, Parpia B, Campbell TC. Dietary calcium and bone density among middle-aged and elderly women in China. Am J Clin Nutr 1993;58:219–27.
40. Matkovic V, Dekanic D, Kostial K. Calcium, teenagers and osteoporosis. In: Roche AF, ed. Osteoporosis: current concepts. Report of the Seventh Ross Conference on Medical Research. Columbus, OH: Ross Laboratories, 1987:64–6.
41. Peterson CA, Eurell JA, Erdman JW. Alterations in calcium intake on peak bone mass in a female rat. J Bone Miner Res 1995;10:81–95.
42. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomized, controlled intervention trial. BMJ 1997;315:1255–60.
43. Chan GM, Hoffman K, McMurray M. Effect of dairy products on bone and body composition in pubertal girls. J Pediatr 1995;126:551–6.
44. Merrilees MJ, Smart EJ, Gilchrist NL, et al. Effects of dairy food supplements on bone mineral density in teenage girls. Eur J Nutr 2000;39: 256–62.
45. Heaney RP. The bone-remodeling transient: implications for the interpretation of clinical studies of bone mass change. J Bone Miner Res 1994;9:1515–23.
46. Slemenda C, Peacock M, Hui S, Zhou L, Johnston CC. Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak bone mass. J Bone Miner Res 1997;12: 676–82.
47. Lee WTK, Leung SSF, Leung DMY, Cheng JCY. A follow-up study on the effects of calcium-supplement withdrawal and puberty on bone acquisition of children. Am J Clin Nutr 1996;64:71–7.
48. Bonjour J-P, Chevalley T, Ammann P, Slosman D, Rizzoli R. Gain in bone mineral mass in prepubertal girls 3–5 years after discontinuation of calcium supplementation: a follow-up study. Lancet 2001;358:1208–12.
49. Dibba B, Prentice A, Ceesay M, et al. Bone mineral contents and plasma osteocalcin concentrations of Gambian children 12 and 24 mo after the withdrawal of a calcium supplement. Am J Clin Nutr 2002;76:681–6.
50. Chan GM, Hess M, Hollis J, Book LS. Bone mineral status in childhood accidental fractures. Am J Dis Child 1984;138:569–70.
51. Goulding A, Cannan R, Williams SM, Gold EJ, Taylor RW, Lewis-Barned NJ. Bone mineral density in girls with forearm fractures. J Bone Miner Res 1998;13:143–8.
52. Landin LA. Fracture patterns in children. Acta Ortho Scand 1983;54: 5S–95S.
53. Bailey DA, Wedge JH, McCulloch, RG, Martin AD, Benhardso SC. Epidemiology of fractures of the distal end of the radius in children as associated with growth. J Bone Joint Surg Am 1989;71:125–30.
54. Khosla S, Melton LJ, Dekutoski MB, Achenbach SJ, Oberg AL, Riggs BL. Incidence of childhood distal forearm fractures over 30 years. A population-based study. JAMA 2003;290:1479–85.

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