See corresponding CME exam on page 1147.

Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10–12-y-old girls: a 2-y randomized trial[1–3]

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ABSTRACT

Background: Little is known about the relative effectiveness of calcium supplementation from food or pills with or without vitamin D supplementation for bone mass accrual during the rapid growth period.

Objective: The purpose was to examine the effects of both food-based and pill supplements of calcium and vitamin D on bone mass and body composition in girls aged 10–12 y.

Design: This placebo-controlled intervention trial randomly assigned 195 healthy girls at Tanner stage I–II, aged 10−12 y, with dietary calcium intakes <900 mg/d to 1 of 4 groups: calcium (1000 mg) + vitamin D_3 (200 IU), calcium (1000 mg), cheese (1000 mg calcium), and placebo. Primary outcomes were bone indexes of the hip, spine, and whole body by dual-energy X-ray absorptiometry and of the radius and tibia by peripheral quantitative computed tomography.

Results: With the use of intention-to-treat or efficacy analysis, calcium supplementation with cheese resulted in a higher percentage change in cortical thickness of the tibia than did placebo, calcium, or calcium + vitamin D treatment ($P = 0.01$, 0.038, and 0.004, respectively) and in higher whole-body bone mineral density than did placebo treatment ($P = 0.044$) when compliance was >50%. With the use of a hierarchical linear model with random effects to control for growth velocity, these differences disappeared.

Conclusions: Increasing calcium intake by consuming cheese appears to be more beneficial for cortical bone mass accrual than the consumption of tablets containing a similar amount of calcium. Diverse patterns of growth velocity may mask the efficacy of supplementation in a short-term trial of children transiting through puberty. *Am J Clin Nutr* 2005;82:1115–26.

KEY WORDS Growth, nutrition intervention, pubertal girls, bone property

INTRODUCTION

Childhood is considered to be a prime time for maximizing one's genetically predetermined peak bone mass through modification of lifestyle and environmental factors. However, the factors governing acquisition of bone mineral content (BMC) and bone mineral density (BMD) as a function of normal growth in childhood are not well understood.

Calcium metabolism during childhood is complex, and the degree of positive calcium balance necessary to achieve maximum peak bone mass is not known. Recent studies have shown that calcium intake and skeletal modeling determine calcium balance during growth and that childhood is a time of high calcium requirements (1, 2). Calcium supplementation intervention studies in children have shown that daily supplementation through either food-based or nonfood calcium supplements can produce significant increases in the percentage gain of bone mass at the lumbar spine and total body but have no effect on BMD of the femoral neck (3). However, no comprehensive evaluations have been carried out of the potential interactions between pubertal status and interventions with respect to bone mass gain. In addition, data are lacking comparing the effectiveness, in terms of bone mass gain in children, of food-based supplements or habitually high calcium intakes with pill supplements and vitamin D supplementation. Calcium pill supplements, as opposed to calcium-rich food supplements, could have different effects on bone mass changes and could operate

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through different mechanisms on bone remodeling processes or on behavioral mediators such as adherence.

A physiologic basis exists supporting a role for other nutrients, such as vitamin D, sodium, phosphorus, vitamin A, vitamin C, potassium, and magnesium, in the utilization of calcium for bone metabolism (4, 5). The importance of adequate vitamin D for calcium absorption and bone metabolism has been well recognized for postmenopausal females. Only recently has there been evidence that vitamin D may be suboptimal in children's diets (6). None of the calcium supplementation studies have evaluated the combined effects of supplementing vitamin D and calcium in children. It is clear that there is a need to evaluate the long-term durability of these effects and the interactions between pubertal development and calcium and vitamin D intakes in terms of bone mass outcomes. The present randomized intervention study was designed to control for the effect of puberty and to examine the effects of both food-based and pill supplements of calcium on bone mass accrual and on body composition in 10–12-y-old girls. In addition, we aimed to examine whether vitamin D supplementation modifies the effectiveness of calcium supplementation.

SUBJECTS AND METHODS

Study design, randomization, and intervention protocol

The study was a 2-y, double-blind (the dairy group was blinded only to the researchers because a nurse gave the girls the supplies), placebo-controlled, randomized intervention trial. All the participants and their legal guardians provided informed consent (volunteers without monetary compensation) in accordance with the ethical committees of the University of Jyväskylä, the Central Hospital of Central Finland, and the Finnish National Agency of Medicines.

The recruitment and randomization process is presented in **Figure 1**. The subjects were first contacted through teachers of 4th to 6th grades in 61 schools in the city of Jyväskylä and its surroundings in Central Finland (96% of the schools in these areas). Of the 1367 girls who participated in the screening, 296 met the inclusion criteria, and 195 (66%) girls and their guardians agreed to participate in the 2-y intervention scheme. The eligibility criteria were no history of serious medical conditions, no history of medication known to affect bone metabolism, sexual development at Tanner stage I to II as determined by a public health nurse using the Tanner grading system (7), age of 10–12 y, and dietary calcium intake less than the Finnish national recommendation of 900 mg/d.

The participants were randomly assigned after their eligibility had been determined at the screening. At first, all eligible subjects were stratified into 2 groups according to their physical activity level as evaluated by questionnaire (*see* the "Data collection" section for details). Assignments were then generated by a computer program in blocks of randomly varying size. Study group assignments were placed in double-sealed envelopes and recorded in a log. The investigators were unblinded at the conclusion of the trial.

The four randomized experimental groups were as follows: *1*) 1000 mg calcium carbonate + 200 IU (5 μg) vitamin D daily (Ca+VD group; $n = 49$); *2*) 1000 mg calcium carbonate daily + vitamin D placebo (Ca group; $n = 49$); *3*) 1000 mg Ca daily from supplemented dairy products (mainly low-fat cheese) (cheese group; $n = 49$); and *4*) calcium placebo + vitamin D placebo identical to the effective pills (placebo group; $n = 48$). After the randomization step, 7 girls informed the researchers that they had asthma, a milk allergy, or used corticosteroids during the baseline assessments. Thus, they were excluded from the initial intervention groups but were provided with active calcium + vitamin D because of ethical considerations. There were also 4 girls who did not want to eat cheese and 4 girls who simply lost interest. Finally, 181 girls who met all the inclusion criteria started the intervention (Ca+VD group, $n = 49$; Ca group, $n = 48$; cheese group, $n = 42$; and placebo group, $n = 42$). In addition, we randomly selected 48 girls from the screening who were at Tanner stage I to II and aged 10–12 y but had habitual dietary calcium intakes >900 mg/d to serve as a reference group.

The supplies used in this study were chewable calcium carbonate tablets (Tums; GlaxoSmithKline, Parsippany, NJ) and swallowing-type calcium carbonate tablets (Kalcipos; Algol Oy, Espoo, Finland). Both tablets had the same calcium (carbonate) content without other minerals. Every 6 mo, the girls chose which type of tablet they would like to use. During the 2-y intervention, 66 girls took chewable calcium carbonate tablets and 54 girls took swallowing-type tablets; the other 32 girls switched from one to another, spending, on average, 12 mo taking chewable tablets and 11.8 mo taking swallowing-type tablets. Vitamin D supplements were water-based drops (Deetipat; 200 IU vitamin D_3/d; Ferrosan Oy, Espoo, Finland). Both calcium and vitamin D placebos were donated by the companies and were identical to the active tablets. Dairy products such as natural low-fat cheese (110 g Edam with 17% fat and 100 g Turunmaa with 15% fat in a daily quantity equivalent to 1000 mg Ca) and lactose-reduced yogurt (Hyla-yogurt for the first 4 mo) were donated by VALIO Oy, Helsinki, Finland. The subjects were advised to take one tablet in the morning and one in the evening. The vitamin D drops were taken in the morning. The dairy products were advised to be taken throughout the day.

Data collection

The girls enrolled in the study were requested to follow a schedule of study visits that included the baseline and follow-up visits at 6, 12, 18, and 24 mo after administration of supplementation. Lifestyle and behavioral characteristics as well as medical history were collected via a self-administered questionnaire at 6-mo intervals. The girls filled out the questionnaire with their parents' assistance, and the questionnaires were checked by a study nurse. Menarcheal age was defined as the first onset of menstrual bleeding and was collected by questionnaire during the trial and through retrospective phone calls after completion of the trail.

The girls' level of physical activity was described in our previous reports (8). Briefly, a self-administrated physical activity questionnaire was used to gather data on the intensity, type, duration, and frequency of leisure-time (after school) physical activity. A physical activity score was calculated as follows:

$$\text{Physical activity score} = \Sigma\,(\text{frequency} \times \text{intensity index} \times \text{duration} \times \text{loading}) \quad (1)$$

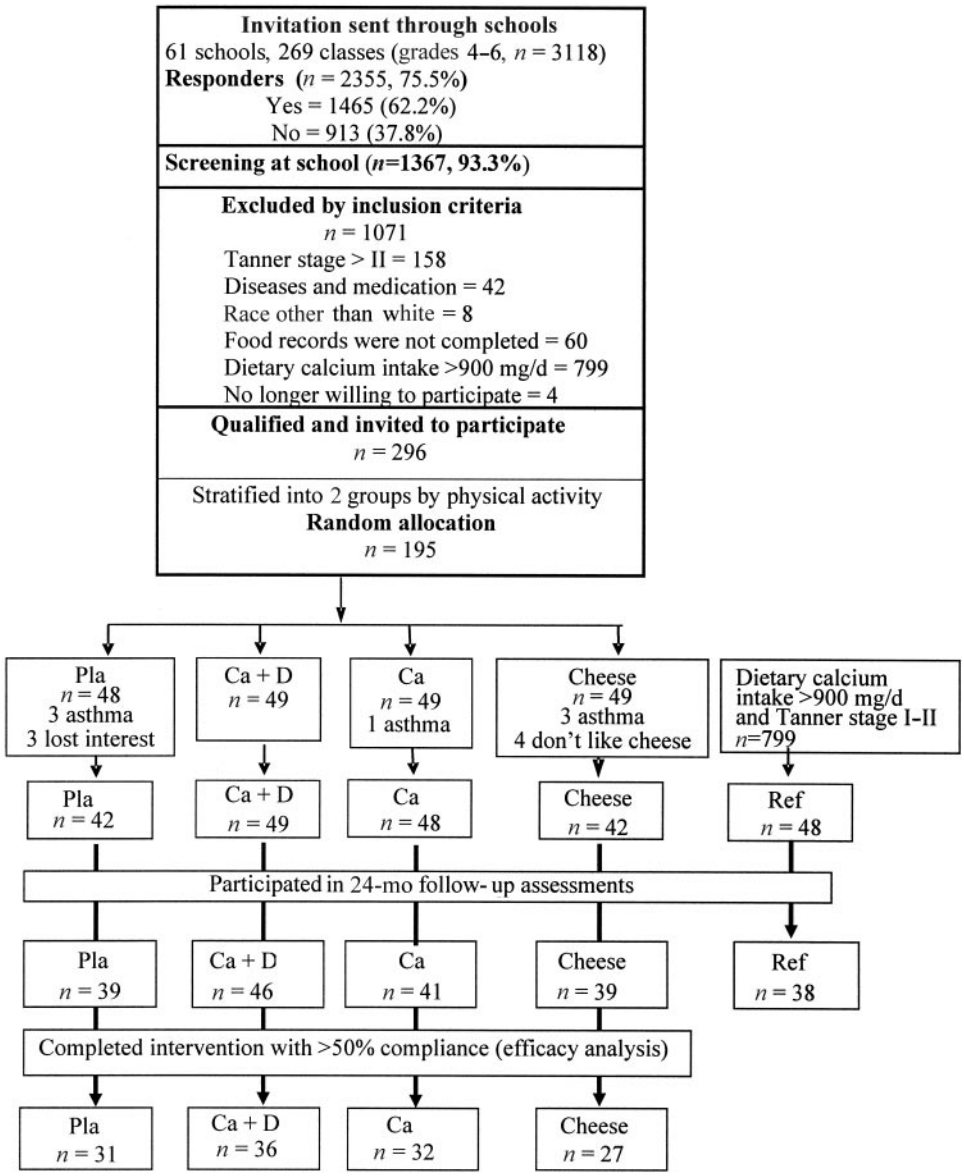

FIGURE 1. Intervention profile. Groups are as follows: Pla, calcium placebo + vitamin D placebo; Ca+D, 1000 mg Ca + 200 IU vitamin D_3; Ca, 1000 mg Ca + vitamin D placebo; cheese, 1000 mg Ca from supplemented dairy products; Ref, reference.

where frequency = times/wk, duration = h/wk, and loading = 1 for non-weight-bearing exercise and 2 for weight-bearing exercise. For the purpose of randomization, the subjects were classified into low and high physical activity groups according to physical activity score (divided by the median value).

Dietary information was obtained from a food-intake diary kept for 3 successive days (2 weekdays, ie, ordinary school days, and 1 weekend day) and is described elsewhere (6). For this report, we analyzed intakes of energy, energy-yielding nutrients, calcium, phosphorus, and vitamin D.

Height and weight were measured with the subjects wearing light clothing and no shoes at 6-mo intervals during the intervention. Body mass index was calculated as weight (kg)/height^2 (m). A public health nurse assessed the Tanner stage of each subject. The Tanner system uses assessments of the pattern of development of pubic hair and breasts. If there was a disagreement between pubic hair and breast, the final decision was made according to the development of the breasts.

Blood samples were taken in the morning between 0730 and 0900 after the subjects had fasted overnight at baseline and after 12 and 24 mo of follow-up. Baseline measurements were taken between the middle of December and the end of February. Each subject had follow-up measurements taken within 2 wk of her anniversary date. If the girls began menstruation, the blood sample was taken between 2 and 5 d after bleeding started. For some of the girls who began menstruation, we took 2 samples for the purpose of matching the annual collection time of the samples for 25-hydroxyvitamin D [25(OH)D] analysis and hormonal analysis. Serum samples were stored at −75 °C until analyzed.

Bone area, BMC, and areal bone mineral density (aBMD) of the whole body, femoral neck, total femur, and lumbar spine (L2–L4) and the lean tissue mass (LTM) and fat mass (FM) of the whole body were assessed at baseline, 12 mo, and 24 mo by using dual-energy X-ray absorptiometry (Prodigy GE; Lunar Corp, Madison, WI). Cross-sectional area (CSA), volumetric BMD (vBMD), and moment of inertia (Ipolar) of the distal radius and the left tibial shaft and cortical thickness of the tibial shaft were measured by using peripheral quantitative computed tomography with an in-plane pixel size of 0.59 mm (pQCT, XCT 2000; Stratec Medizintechnik, GmbH, Pforzheim, Germany) (6, 8). The precision of repeated measurements was expressed by the percentage CV. The CV ranged from 0.6% to 1.2% for BMC and from 0.9% to 1.3% for aBMD of the femoral neck, total femur, L2–L4, and whole body; CV was 1.0% for LTM and 2.2% for FM of the whole body. The results for CSA, vBMD, cortical thickness, and Ipolar of the distal radius and the left tibial shaft were analyzed by using a validated software program (GEANIE; BonAlyse Oy, Jyväskylä, Finland) as described elsewhere (8). The CV between 2 consecutive measurements with repositioning varied between 1.1% and 3.0% for CSA and was <1% for vBMD at the different bone sites.

Calcitropic hormones, leptin, bone resorption and formation markers, and calcium excretion were secondary outcomes. Serum 25(OH)D and intact parathyroid hormone (iPTH) concentrations were measured by enzyme immunoassay (Immunodiagnostic Systems Ltd, Boldon, United Kingdom). The intra- and interassay CVs for 25(OH)D were 4.2% and 6.1%, and those for iPTH were 2.9% and 4.9%, respectively. The serum leptin concentration was assessed by using an enzyme immunoassay (DSL-10-23100 ACTIVE Human Leptin Enzyme-Linked Immunosorbent; Diagnostic Systems Laboratories Inc, Webster, TX). The intra- and interassay CVs were 3.8% and 4.4%, respectively.

Twenty-four–hour urine samples were collected to assess calcium excretion by colorimetric assay photometry (iEMS reader MF; Thermo Labsystems Oy, Vantaa, Finland) (9). Urinary calcium excretion was adjusted for urine volume, hours of collection, and creatinine excretion. The intra- and interassay CVs were 3.2% and 1.1%, respectively. Urine samples were stored at −20 °C until analyzed. All the biomarkers and hormones were analyzed blindly and were assessed randomly with respect to the time the sample was taken and the intervention group assigned.

Statistical analysis

The trial was designed with adequate power (90%; α level of 0.05) to detect a 3–4% difference between the treatment groups and the control group in the primary outcome of BMC over the 2-y period. All data collected by questionnaire were entered into database management software (FILEMAKER PRO version 7; FileMaker Inc, Santa Clara, CA), double-checked by independent researchers, and exported to SPSS software (version 12; SPSS Inc, Chicago, IL) for statistical analysis. Bone properties and biomarker data were directly transferred (electronically) to SPSS and were checked by researchers against the original data. All data were checked for normality by using the Shapiro-Wilk test in SPSS 12.0 for WINDOWS. If the data were not normally distributed, they were transformed before further analysis. Descriptive statistics were used to present the anthropometric data and bone information for girls at the baseline and 24-mo assessments as means and 95% CIs unless otherwise stated.

An intention-to-treat analysis was performed to compare the Ca+D, Ca, and cheese groups with the placebo group. The effects of the interventions were assessed by using analysis of covariance (ANCOVA) for repeated measures (treatment group × time) with baseline Tanner stage as a covariate. If the significance of the group-by-time interaction was $P < 0.05$, the effect was localized by using Bonferroni's correction for multiple comparisons. The level of statistical significance chosen for the contrasts was $P < 0.05$. In addition to the intention-to-treat analysis, efficacy or active treatment analysis was done when supplements were taken for ≥50% of the whole trial and for <50% but not >3 mo continuously [ANCOVA for repeated measures (treatment group × time) with baseline Tanner stage as a covariate]. The percentage differences (0–24 mo) were calculated from duration between baseline and endpoint measurements for each individual. The comparison of percentage changes in different groups was performed by using ANCOVA (2-factor interactions: compliance or noncompliance × treatment group) controlled for the baseline value and Tanner stage and with use of Bonferroni's correction for multiple comparisons. If the significance of the overall group difference was $P < 0.05$, then the effect was localized by contrast with the placebo group. When the 95% CI did not include zero, the difference was regarded as statistically significant at $\alpha = 0.05$.

A hierarchical linear model with random effects was used to explore the growth patterns of body height, weight, LTM, FM, bone area, BMC, and BMD of the total body during puberty. In this model, the time relative to menarche was entered as the explanatory variable in the form of polynomial spline functions to explain the change in these variables over time in different intervention groups (MLwiN 1.1 software; Multiple Project, Institute of Education, University of London, London, United Kingdom). The growth velocity of different variables was deduced by differentiation of its corresponding model with regard to time relative to menarche.

No comparisons were made between the reference group and the other groups. The results of the reference group were used as a reference and were evaluated statistically only in the hierarchical linear model.

RESULTS

Baseline characteristics

The characteristics of the study groups at baseline are given in **Table 1**. There were no significant group differences with respect to physical characteristics or level of physical activity at baseline, except for Tanner stage ($P = 0.027$). The Ca+D group had more girls at Tanner stage I than did the other groups.

Twenty-five girls did not reach menarche until the end of the measurements, and 19 girls did not report their age at the start of menarche. The average age at the start of menarche was 12.9 y (95% CI: 11.5, 14.3 y). The average body height at the start of menarche was 157.4 cm (95% CI: 144.8, 169.9 cm), average body weight was 48.9 kg (95% CI: 32.5, 65.3 kg), average LTM was 34.3 kg (95% CI: 27.1, 41.4 kg), and average FM was 12.0 kg (95% CI: 4.1, 35.0 kg). No significant differences in age or body height at the start of menarche were found between the intervention groups.

TABLE 1
Baseline characteristics of the randomly assigned groups and the reference group[1]

Variable	Placebo group ($n = 48$)	Ca+D group ($n = 49$)	Ca group ($n = 49$)	Cheese group ($n = 49$)	Reference group ($n = 48$)
Age (y)	11.3 ± 0.7[2]	11.0 ± 0.6	11.2 ± 0.8	11.2 ± 0.8	11.3 ± 0.7
Height (cm)	145.5 ± 8.1	142.8 ± 7.9	146.5 ± 8.0	144.4 ± 7.5	147.2 ± 6.4
Weight (kg)	38.5 ± 9.5	37.5 ± 9.2	40.8 ± 9.0	37.2 ± 7.0	40.0 ± 7.7
BMI (kg/m^2)	18.0 ± 3.2	18.2 ± 3.1	18.8 ± 2.7	17.8 ± 2.6	18.4 ± 2.7
Tanner stage (%)					
I	53	73	44	57	42
II	47	27	56	43	58
Exercise					
(times/wk)	2.1 ± 1.1	2.6 ± 1.9	2.3 ± 1.8	2.4 ± 1.6	3.1 ± 2.1
(h/wk)	2.5 ± 1.9	2.7 ± 2.2	3.1 ± 2.1	2.7 ± 1.9	2.9 ± 2.0
Energy (MJ/d)	6.1 ± 1.2	6.3 ± 1.6	6.0 ± 1.5	6.2 ± 1.4	7.8 ± 1.2
Protein (% of energy)	15.0 ± 3.0	14.3 ± 2.5	14.9 ± 3.0	14.9 ± 2.5	16.5 ± 2.0
Fat (% of energy)	31.9 ± 5.5	32.7 ± 6.1	30.7 ± 5.2	32.0 ± 6.3	31.5 ± 5.4
Carbohydrates (% of energy)	53.1 ± 6.7	53.0 ± 7.3	54.4 ± 6.6	53.1 ± 6.1	52.0 ± 6.2
Calcium (mg/d)	671 ± 135	664 ± 191	667 ± 171	680 ± 183	1351 ± 323
Phosphorus (mg/d)	993 ± 169	987 ± 224	1003 ± 208	1009 ± 161	1548 ± 263
Vitamin D (μg/d)	2.4 ± 1.5	2.7 ± 1.9	2.5 ± 1.8	2.4 ± 1.4	3.7 ± 2.2

[1] Placebo group, calcium placebo + vitamin D placebo; Ca+D group, 1000 mg Ca + 200 IU vitamin D_3; Ca group, 1000 mg Ca + vitamin D placebo; cheese group, 1000 mg Ca from supplemented dairy products. There were no significant differences among the 4 treatment groups (ANOVA) except for Tanner stage, which was significant at $P = 0.027$ (chi-square test).
[2] $\bar{x}$ ± SD (all such values).

The mean value of 25(OH)D was 45.9 nmol/L (95% CI: 43.8, 48.0 nmol/L). The corresponding values for iPTH and leptin were 24.6 pg/L (95% CI: 23.3, 26.0 pg/L) and 19.4 ng/mL (95% CI: 17.0, 22.1 ng/mL).

Intervention adherence and compliance

Two hundred three girls (83%) participated in the endpoint measurements (Figure 1). The main reasons for dropping out or not completing the intervention were as follows: lack of time or interest ($n = 11$, either children or family), onset of disease not related to this trial ($n = 4$), dislike tablets ($n = 23$), dislike cheese ($n = 12$), no special reason ($n = 2$), and moved away ($n = 1$). Of the girls enrolled in the trial ($n = 195$), we have follow-up data on 188 (96.4%) at 6 mo, 180 (92.3%) at 12 mo, 174 (89.2%) at 18 mo, and 173 (88.7%) at 24 mo. The duration of the intervention was similar for the intervention groups (24.0 ± 0.5 mo for the placebo group, 24.0 ± 0.6 mo for the Ca+D group, 24.1 ± 0.7 mo for the Ca group, and 23.8 ± 0.9 mo for the cheese group). In addition, we had data on 38 (79.2%) girls in the reference group at 24 mo.

Compliance information was obtained every 3 mo from 2 sources. A special diary was provided for the girls to document their intakes of the study calcium tablets, vitamin D drops, and cheese products. Compliance was also obtained by counting the returned tablets every 3 mo. A study coordinator contacted the girls and their parents by phone frequently to check on compliance. If there was a discrepancy, the study coordinator would double-check the results with the participant's family to clarify compliance.

Overall compliance with calcium, placebo, or cheese intake was 70% for the Ca+D group, 68% for the Ca group, 63% for the cheese group, and 72% for the placebo group. Compliance with vitamin D or placebo drops was 77% for the Ca+D group, 71% for the Ca group, and 81% for the placebo group. At baseline, mean calcium intake was 643 mg/d for the Ca+D group, 679 mg/d for the Ca group, 706 mg/d for the cheese group, 664 mg/d for the placebo group, and 1320 mg/d for the reference group. Vitamin D intakes were 2.8, 2.6, 2.5, 2.3, and 3.6 μg/d in the Ca+D, Ca, cheese, placebo, and reference groups, respectively. The mean calcium intake (supply + diet) for the entire trial was 1674 mg/d for the Ca+D group, 1566 mg/d for the Ca group, 1413 mg/d for the cheese group, 843 mg/d for the placebo group, and 1269 mg/d for the reference group. The corresponding figures for vitamin D intake were 6.5, 3.1, 3.0, 2.7, and 4.0 μg/d, respectively. Vitamin D intake exceeded the recommendation level during the trial (5 μg/d) only in the Ca+D group.

Changes in bone indexes (ANCOVA with repeated measures)

Intention-to-treat analysis

No overall significant differences (group × time interaction) were found in iPTH, leptin, or markers of bone turnover between the groups. 25(OH)D concentrations increased 18% ($P < 0.01$) in the Ca+D group compared with a decline in the Ca (−10%), cheese (−11%), and placebo (−20%) groups. The Ca excretion level was elevated in all groups with time ($\bar{x} = 57.0$ mg/d at baseline, 85.6 at 6 mo, 70.0 at 12 mo, 84.1 at 18 mo, and 75.9 at 24 mo, respectively), but did not show significant group-by-time differences.

No significant interactions of group by time in changes in body weight, height, FM, LTM, bone area, BMC, or aBMD of the whole body were found (**Table 2**). Similar results were observed for the femoral neck, total femur, and L2–L4 as measured by dual-energy X-ray absorptiometry (**Table 3**).

No significant differences in the changes were found in any of the variables measured by pQCT except for cortical thickness of the tibia ($P = 0.001$; **Table 4**). A significant contrast with the placebo group was not observed.

TABLE 2
Anthropometric and whole-body variables measured by dual-energy X-ray absorptiometry in the intervention groups (intention-to-treat analysis) and in the reference group[1]

	Placebo group (n = 39)		Ca+D group (n = 46)		Ca group (n = 41)		Cheese group (n = 39)		Reference group (n = 38)	
Variable	Baseline value	Change	Baseline value	Change	Baseline value	Change	Baseline value	Change	Baseline value	Change
		%		%		%		%		%
Height (cm)	146 ± 7.5	8.7 ± 0.3	143 ± 8.1	8.4 ± 0.3	146 ± 7.3	8.7 ± 0.3	144 ± 8.3	9.1 ± 0.3	147 ± 6.4	9.1 ± 0.3
Weight (kg)	38.0 ± 7.1	30.5 ± 1.6	37.5 ± 9.4	28.3 ± 1.4	40.1 ± 8.7	29.8 ± 1.5	37.0 ± 7.2	31.1 ± 1.6	40.0 ± 7.7	28.3 ± 1.6
FM (kg)	9.7 ± 4.5	37.7 ± 4.5	10.1 ± 6.0	35.2 ± 4.2	11.1 ± 5.6	35.3 ± 4.5	10.0 ± 4.8	34.2 ± 4.5	10.7 ± 5.4	28.5 ± 4.9
LTM (kg)	27.0 ± 3.8	28.1 ± 1.3	25.9 ± 4.3	26.5 ± 1.2	27.8 ± 4.3	28.8 ± 1.3	25.8 ± 3.5	29.6 ± 1.3	27.7 ± 3.4	30.2 ± 1.4
BA (cm^2)	1465 ± 213	24.0 ± 1.0	1410 ± 221	22.7 ± 0.9	1506 ± 210	23.0 ± 1.0	1430 ± 119	25.1 ± 1.0	1508 ± 157	24.4 ± 1.1
BMC (g)	1383 ± 257	35.0 ± 1.4	1328 ± 292	34.7 ± 1.3	1431 ± 274	35.0 ± 1.4	1343 ± 235	38.1 ± 1.4	1437 ± 216	36.9 ± 1.4
aBMD (g/cm^2)	0.939 ± 0.1	8.9 ± 0.5	0.934 ± 0.1	9.8 ± 0.5	0.945 ± 0.1	9.7 ± 0.5	0.935 ± 0.1	10.4 ± 0.5	0.949 ± 0.1	10.2 ± 0.6

[1] All values are $\bar{x}$ ± SD. Estimated percentage changes over the 2-y period are controlled for baseline value and Tanner stage. FM, fat mass; LTM, lean tissue mass; BA, bone area; BMC, bone mineral content; aBMD, area bone mineral density. Intervention groups are as defined in Table 1. Follow-up data were not given, but the statistical analysis (intention-to-treat) was performed with use of analysis of covariance (ANCOVA) for repeated measures (2-factor interactions: treatment group × time) with baseline Tanner stage as a covariate. No interaction was found between the groups. The comparison of percentage changes in the different groups was performed by using ANCOVA (2-factor interactions: compliance or noncompliance × treatment group) controlled for the baseline value and Tanner stage. No interaction was found between the groups except that for aBMD (P = 0.008). aBMD of the whole body increased more in the cheese group with compliance >50% than in the placebo group (P = 0.044, Bonferroni corrected for multiple comparisons).

Efficacy analysis

The efficacy analysis yielded significant overall differences (group × time) in the changes in BMC in the whole body (P = 0.053), total femur (P = 0.040), and L2–L4 (P = 0.044), as well as in aBMD in the whole body (P = 0.013) and total femur (P = 0.012). No specific group difference was found as the result of larger interindividual variations (data not shown). No significant interaction of group by time was found in the pQCT-measured variables except for cortical thickness of the tibia (P = 0.002), but no specific group difference by contrast with the placebo group was found.

Percentage changes in bone indexes

Bone properties increased significantly in all groups over the 2-y period (Tables 2, 3, and 4). The average percentage change in BMC measured by dual-energy X-ray absorptiometry from baseline to 24 mo in the different groups versus the placebo group is shown in **Figure 2**. Neither the intention-to-treat nor the efficacy analyses showed any significant differences between the groups for BMC at any measured bone site. Interestingly, the efficacy analysis yielded significant overall differences in the changes in whole-body aBMD (P = 0.008), and the cheese group with compliance >50% gained more than did the placebo group (P =

TABLE 3
Bone variables measured by dual-energy X-ray absorptiometry in the intervention groups (intention-to-treat analysis) and in the reference group[1]

	Placebo group (n = 39)		Ca+D group (n = 46)		Ca group (n = 41)		Cheese group (n = 39)		Reference group (n = 38)	
Variables	Baseline value	Change	Baseline value	Change	Baseline value	Change	Baseline value	Change	Baseline value	Change
		%		%		%		%		%
BA_{FN} (cm^2)	3.82 ± 0.6	15.0 ± 1.6	3.82 ± 0.5	12.6 ± 1.5	3.76 ± 0.6	14.9 ± 1.6	3.77 ± 0.5	13.1 ± 1.6	3.96 ± 0.4	11.1 ± 2.3
BMC_{FN} (g)	3.23 ± 0.5	22.4 ± 1.5	3.07 ± 0.5	24.0 ± 1.4	3.34 ± 0.5	23.3 ± 1.5	3.16 ± 0.4	26.5 ± 1.4	3.33 ± 0.5	26.1 ± 1.6
$aBMD_{FN}$ (g/cm^2)	0.806 ± 0.1	12.9 ± 1.0	0.779 ± 0.1	14.2 ± 1.0	0.818 ± 0.1	14.5 ± 1.0	0.815 ± 0.1	14.8 ± 1.0	0.839 ± 0.1	13.9 ± 1.1
BA_{TF} (cm^2)	23.9 ± 2.9	17.3 ± 0.8	23.2 ± 3.0	17.0 ± 0.7	24.1 ± 2.6	17.8 ± 0.98	23.0 ± 2.6	18.1 ± 0.8	24.1 ± 1.8	17.4 ± 0.9
BMC_{TF} (g)	19.6 ± 3.9	33.6 ± 1.6	18.9 ± 4.1	33.6 ± 1.6	20.3 ± 3.8	36.4 ± 1.7	18.9 ± 3.2	36.9 ± 1.6	20.6 ± 3.2	34.8 ± 1.7
$aBMD_{TF}$ (g/cm^2)	0.818 ± 0.1	14.1 ± 1.0	0.805 ± 0.1	14.2 ± 1.0	0.838 ± 0.1	15.1 ± 1.0	0.821 ± 0.1	15.5 ± 1.0	0.852 ± 0.1	14.9 ± 1.0
BA_{L2-4} (cm^2)	27.7 ± 3.7	23.4 ± 0.9	26.9 ± 3.6	23.0 ± 0.9	28.4 ± 3.5	24.4 ± 0.9	26.9 ± 3.7	25.3 ± 0.9	28.5 ± 3.1	26.6 ± 1.0
BMC_{L2-4} (g)	23.1 ± 5.6	47.0 ± 2.2	21.8 ± 5.8	46.9 ± 2.0	24.0 ± 5.7	48.9 ± 2.2	22.2 ± 5.6	52.4 ± 2.2	23.6 ± 4.3	55.0 ± 2.3
$aBMD_{L2-4}$ (g/cm^2)	0.822 ± 0.1	19.0 ± 1.1	0.800 ± 0.1	19.2 ± 1.0	0.837 ± 0.1	19.4 ± 1.1	0.813 ± 0.1	21.5 ± 1.1	0.823 ± 0.1	22.5 ± 1.1

[1] All values are $\bar{x}$ ± SD. Estimated percentage changes over the 2-y period are controlled for baseline value and Tanner stage. BA, bone area; BMC, bone mineral content; aBMD, area bone mineral density; FN, femoral neck; TF, total femur; L2–4, lumbar spine 2–4. Intervention groups are as defined in Table 1. Follow-up data were not given, but the statistical analysis (intention-to-treat) was performed with use of analysis of covariance (ANCOVA) for repeated measures (2-factor interactions: treatment group × time) with baseline Tanner stage as a covariate. No interaction was found between the groups. The comparison of percentage changes in the different groups was performed by using ANCOVA (2-factor interactions: compliance or noncompliance × treatment group) controlled for the baseline value and Tanner stage. No interaction was found between the groups except that for aBMD of the TF (P = 0.009). No significant difference between the intervention groups and the placebo group was found (Bonferroni corrected for multiple comparisons).

TABLE 4
Bone variables measured by peripheral quantitated computed tomography in the intervention groups (intention-to-treat analysis) and in the reference group[1]

	Placebo group (n = 39)		Ca+D group (n = 46)		Ca group (n = 41)		Cheese group (n = 39)		Reference group (n = 38)	
Variable	Baseline value	Change	Baseline value	Change	Baseline value	Change	Baseline value	Change	Baseline value	Change
		%		%		%		%		%
CSA_{radius} (mm^2)	224 ± 37	21.3 ± 2.0	212 ± 45	23.0 ± 1.9	234 ± 46	26.0 ± 2.1	220 ± 39	26.2 ± 2.0	228 ± 40	26.1 ± 2.2
BMC_{radius} (mg/mm)	63.0 ± 8.0	22.2 ± 2.0	60.2 ± 12.2	22.6 ± 1.8	64.5 ± 10.1	24.4 ± 2.0	63.8 ± 11.0	25.9 ± 1.9	65.3 ± 8.9	25.5 ± 1.9
$vBMD_{radius}$ (mg/cm^3)	286 ± 41	1.99 ± 1.5	287 ± 37	3.35 ± 1.4	283 ± 39	2.61 ± 1.5	293 ± 32	3.07 ± 1.5	291 ± 36	0.84 ± 1.5
$Ipolar_{radius}$ (mg · cm)	278 ± 67	51.8 ± 4.2	256 ± 94	53.3 ± 4.1	304 ± 104	62.0 ± 4.4	281 ± 90	61.8 ± 4.2	296 ± 79	62.4 ± 4.3
CSA_{tibia} (mm^2)	277 ± 37	14.8 ± 1.1	274 ± 41	15.6 ± 1.0	283 ± 37	15.8 ± 1.1	271 ± 36	15.9 ± 1.1	291 ± 36	14.4 ± 1.2
BMC_{tibia} (mg/mm)	235 ± 32	22.7 ± 1.0	234 ± 39	23.0 ± 0.9	238 ± 29	24.3 ± 1.0	232 ± 31	25.2 ± 1.0	249 ± 34	22.6 ± 1.0
$vBMD_{tibia}$ (mg/cm^3)	852 ± 42	7.76 ± 0.6	855 ± 44	6.87 ± 0.5	855 ± 35	7.53 ± 0.6	856 ± 33	8.30 ± 0.6	855 ± 40	8.76 ± 0.6
Cth_{tibia} (mm)	2.80 ± 0.4	31.1 ± 1.4	2.85 ± 0.5	31.7 ± 1.3	2.79 ± 0.3	29.8 ± 1.4	2.75 ± 0.3	37.1 ± 1.3	2.97 ± 0.5	32.7 ± 1.5
$Ipolar_{tibia}$ (mg · cm)	1907 ± 57.2	39.7 ± 2.3	1803 ± 536	41.3 ± 2.2	2056 ± 634	41.5 ± 2.3	1825 ± 537	42.6 ± 2.3	2064 ± 507	43.6 ± 2.3

[1] All values are $\bar{x}$ ± SD. Estimated percentage changes over the 2-y period are controlled for baseline value and Tanner stage. CSA, bone cross-sectional area; BMC, bone mineral content; vBMD, volumetric bone mineral density; Cth, cortical bone thickness; Ipolar, polar moment of inertia. Intervention groups are as defined in Table 1. Follow-up data were not given, but the statistical analysis (intention-to-treat) was performed with use of analysis of covariance (ANCOVA) for repeated measures (2-factor interacti ons: treatment group × time) with baseline Tanner stage as a covariate. No interaction was found between the groups except that for Cth ($P = 0.014$, Bonferroni corrected for multiple comparisons), but no group difference by contrast with the placebo group was found. The comparison of percentage changes in different groups was performed by using ANCOVA (2-factor interactions: compliance or noncompliance × treatment group) controlled for the baseline value and Tanner stage. No interaction was found between the groups except that for Cth ($P = 0.049$). Cth of the tibia increased more in the cheese group with compliance >50% than in the placebo ($P = 0.01$), Ca+D ($P = 0.004$), or Ca ($P = 0.038$) group (Bonferroni corrected for multiple comparisons).

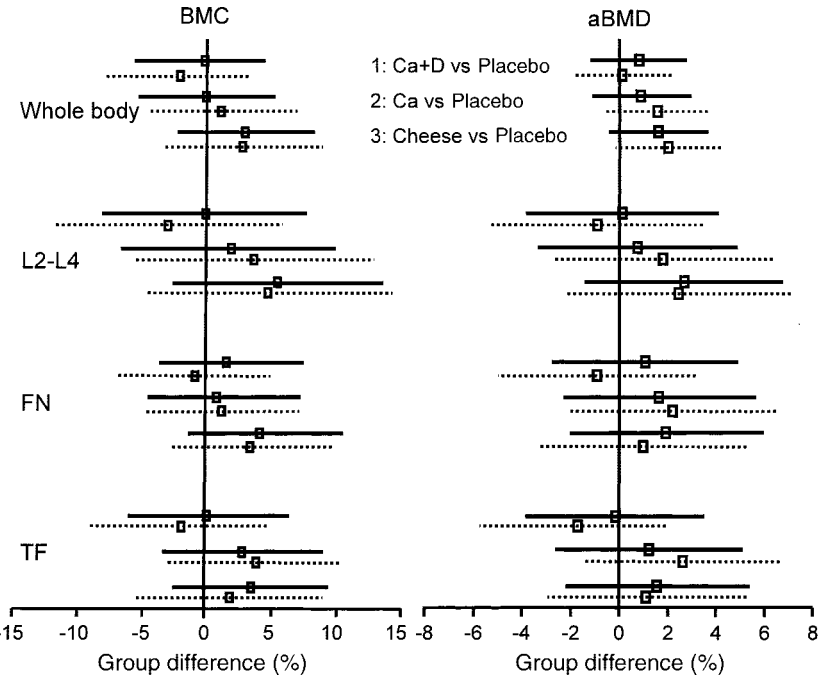

FIGURE 2. Mean (95% CI) group differences (adjusted for baseline value and Tanner stage) in bone mineral content (BMC) and areal bone mineral density (aBMD) of the whole body, lumbar spine (L2-L4), femoral neck (FN), and total femur (TF). The solid lines represent the intention-to-treat analysis and the dotted lines the efficacy analysis. The lines follow the order (from top to bottom) 1: Ca+D versus placebo; 2: Ca versus placebo; and 3: cheese versus placebo. Pla group, calcium placebo + vitamin D placebo; Ca+D group, 1000 mg Ca + 200 IU vitamin D_3; Ca group, 1000 mg Ca + vitamin D placebo; cheese group, 1000 mg Ca from supplemented dairy products The comparison of percentage changes in different groups was performed by using analysis of covariance (2-factor interactions: compliance or noncompliance × treatment group) controlled for baseline value and Tanner stage. No interaction was found between the groups expect that for aBMD of the whole body ($P = 0.008$). aBMD of the whole body increased more in the cheese group with compliance >50% (efficacy analysis) than in the placebo group ($P = 0.044$, Bonferroni corrected for multiple comparisons). There was also an overall interaction for aBMD of the TF ($P = 0.009$). However, no significant difference between the intervention groups and the placebo group was found.

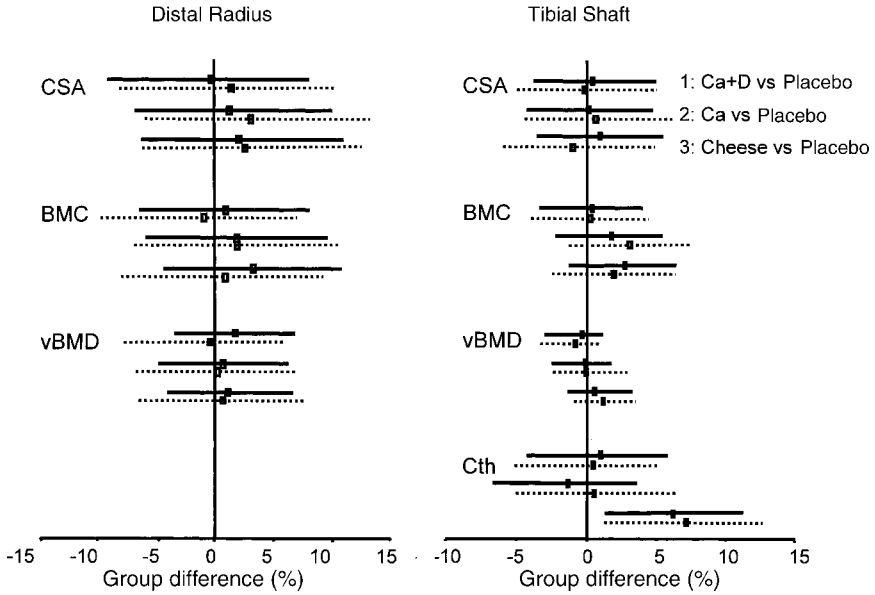

FIGURE 3. Mean (95% CI) group differences (adjusted for baseline value and Tanner stage) in cross-sectional area (CSA), bone mineral content (BMC), and volumetric bone mineral density (vBMD) of the distal radius and tibial shaft and cortical thickness (Cth) of the tibia as measured by peripheral quantitative computed tomography. The solid lines represent the intention-to-treat analysis and the dotted lines the efficacy analysis. The lines follow the order (from top to bottom) 1: Ca+D versus placebo; 2: Ca versus placebo; and 3: cheese versus placebo. Pla group, calcium placebo + vitamin D placebo; Ca+D group, 1000 mg Ca + 200 IU vitamin D_3; Ca group, 1000 mg Ca + vitamin D placebo; cheese group, 1000 mg Ca from supplemented dairy products. The comparison of percentage changes in different groups was performed by using analysis of covariance (2-factor interactions: compliance or noncompliance × treatment group) controlled for baseline value and Tanner stage. No interaction was found between the groups except that for Cth ($P = 0.049$). Cth of the tibia increased more in the cheese group with compliance >50% (efficacy analysis) than in the placebo ($P = 0.01$), Ca+D ($P = 0.004$), or Ca ($P = 0.038$) group (Bonferroni corrected for multiple comparisons).

0.044, Figure 2). There was also an overall interaction for aBMD of the total femur ($P = 0.009$). However, no significant difference between the intervention groups by contrast with the placebo group was found (Table 3 and Figure 2).

The percentage changes in CSA, BMC, vBMD, and cortical thickness measured by pQCT are shown in **Figure 3**. There were large interindividual variations, and only cortical thickness of the tibia showed significant overall group differences ($P = 0.049$). The cheese group with compliance >50% increased more by contrast with the placebo ($P = 0.01$), Ca+D ($P = 0.004$), and Ca ($P = 0.038$) groups (Bonferroni corrected for multiple comparisons). This significance remained even in the intention-to-treat analysis ($P = 0.01$, $P = 0.024$, and $P = 0.001$ for the cheese group versus the placebo, Ca+D and the Ca groups, respectively).

Growth and intervention

With the use of a multilevel analysis taking into account the differences in peak growth speed, no significant group differences were observed in any of the analyzed variables (**Figure 4** and **Figure 5**; results of the whole body only are shown). All groups had a similar growth velocity and pattern with respect to changes in bone mass and body composition. Thus, all groups were pooled to calculate the growth velocities of different variables. Before menarche, the growth velocities (rate of change with time) peaked at 16.7 mo (11.5 y old) for height, at 9.1 mo (12.1 y old) for body weight, at 13.5 mo (11.8 y old) for LTM, and at 17.7 mo (11.4 y old) for FM (Figure 4). The magnitudes of peak growth velocities were 0.6 cm/mo for height, 0.5 kg/mo for weight, 0.2 kg/mo for LTM, and 0.1 kg/mo for FM. The rate of change with time of whole-body bone area peaked at 12.9 mo (11.8 y old) and that of BMC peaked at 3.1 mo (12.6 y old) before menarche; the highest rates were 15.9 cm^2/mo and 22.5 g/mo, respectively. On the other hand, the rate of change with time of whole-body BMD peaked at 7.3 mo (13.5 y old) after menarche, with a peak rate of 4.9 $g \cdot cm^{-2} \cdot mo^{-1}$ (Figure 5).

DISCUSSION

In this 2-y, double-blind (except for cheese), placebo-controlled, randomized intervention trial, we found that adequate calcium intake from food is more beneficial for bone mass accrual than is the use of tablets containing a similar amount of calcium. Girls in the cheese group tended to gain more bone than did the girls in the other groups, but the change was significant ($P = 0.01$) only for cortical thickness of the tibia, with an increase in whole-body aBMD observed compared with the placebo group when compliance was >50% ($P = 0.044$). However, when taking into account the differences in growth and maturation of different individuals, no significant differences in bone gain were observed in any of the measured bone sites and variables, nor were there differences in the development of body composition between the intervention groups (Figures 4 and 5). It is important to recognize that the effect of supplementation may have been masked by the diverse growth patterns in our sample.

Our study is the first randomized trial of calcium supplementation in 2 forms (pill and cheese) and vitamin D supplementation in children to also include a reference group with habitual high calcium intakes. The possible benefits of cheese and habitual high calcium intakes from food over the pills may be a result of: *1*) a better absorption of calcium from dairy products as a result of the presence of lactose or caseinphosphopeptides (10); *2*) ideal

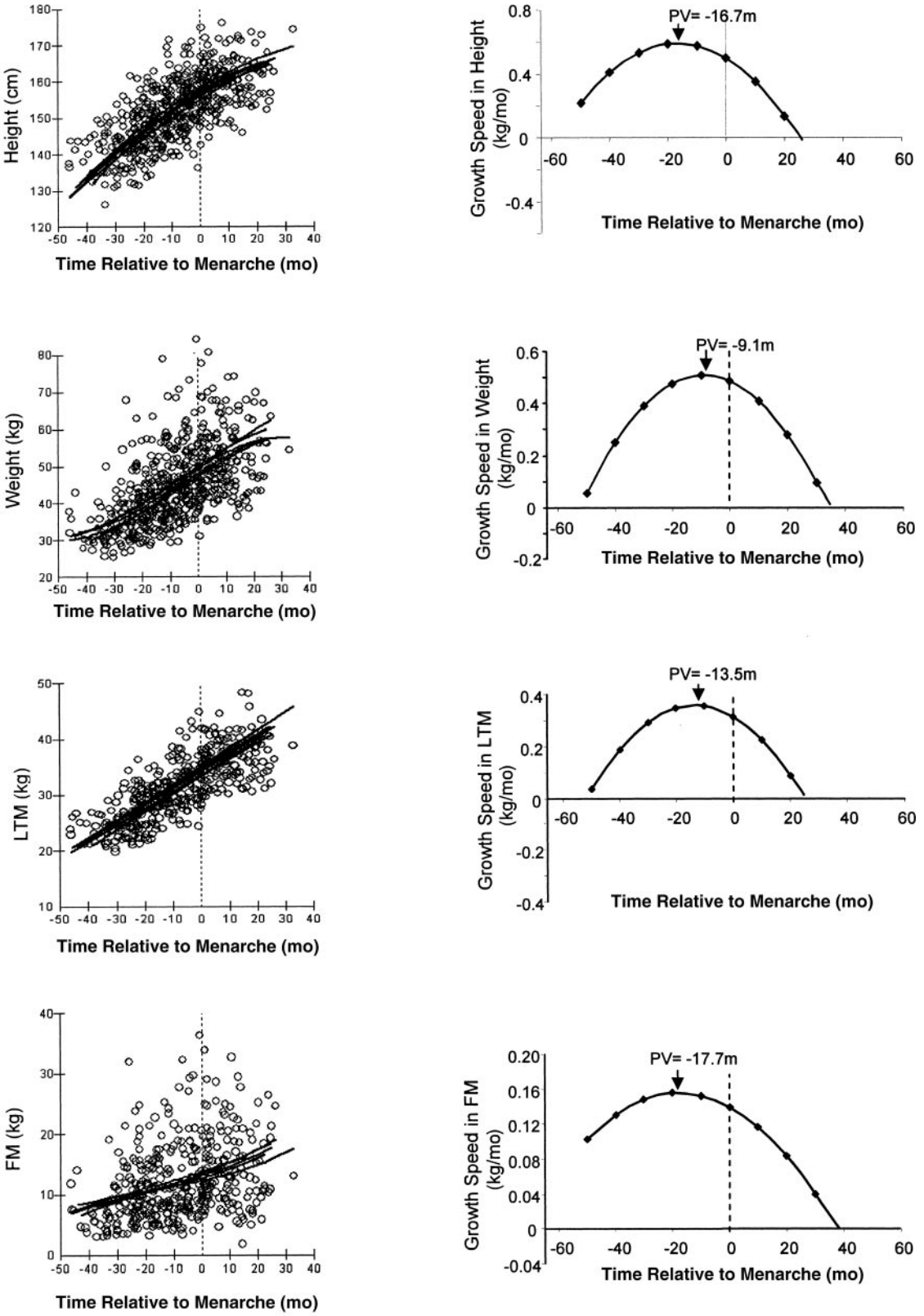

FIGURE 4. Growth curves of body height, weight, lean tissue mass (LTM), and fat mass (FM). In the left panel, each circle indicates an individual value. The lines represent the hierarchical linear model with random effects for different groups. Because no significant group differences were found, the growth speed curves in the right panel are for the total sample. The best-fitting lines were as follows: Body height (cm) = $157.4 + 0.497 \times t - 5.63 \times 10^{-3} \times t^2 - 1.12 \times 10^{-4} \times t^3$; body weight (kg) = $48.9 + 0.484 \times t - 2.451 \times 10^{-3} \times t^2 - 0.9 \times 10^{-4} \times t^3$; LTM (kg) = $34.3 + 0.313 \times t - 3.24 \times 10^{-3} \times t^2 - 0.8 \times 10^{-4} \times t^3$; and FM (kg) = $13.2 + 0.140 \times t - 0.899 \times 10^{-3} \times t^2 - 0.2 \times 10^{-4} - t^3$, where t is time in mo.

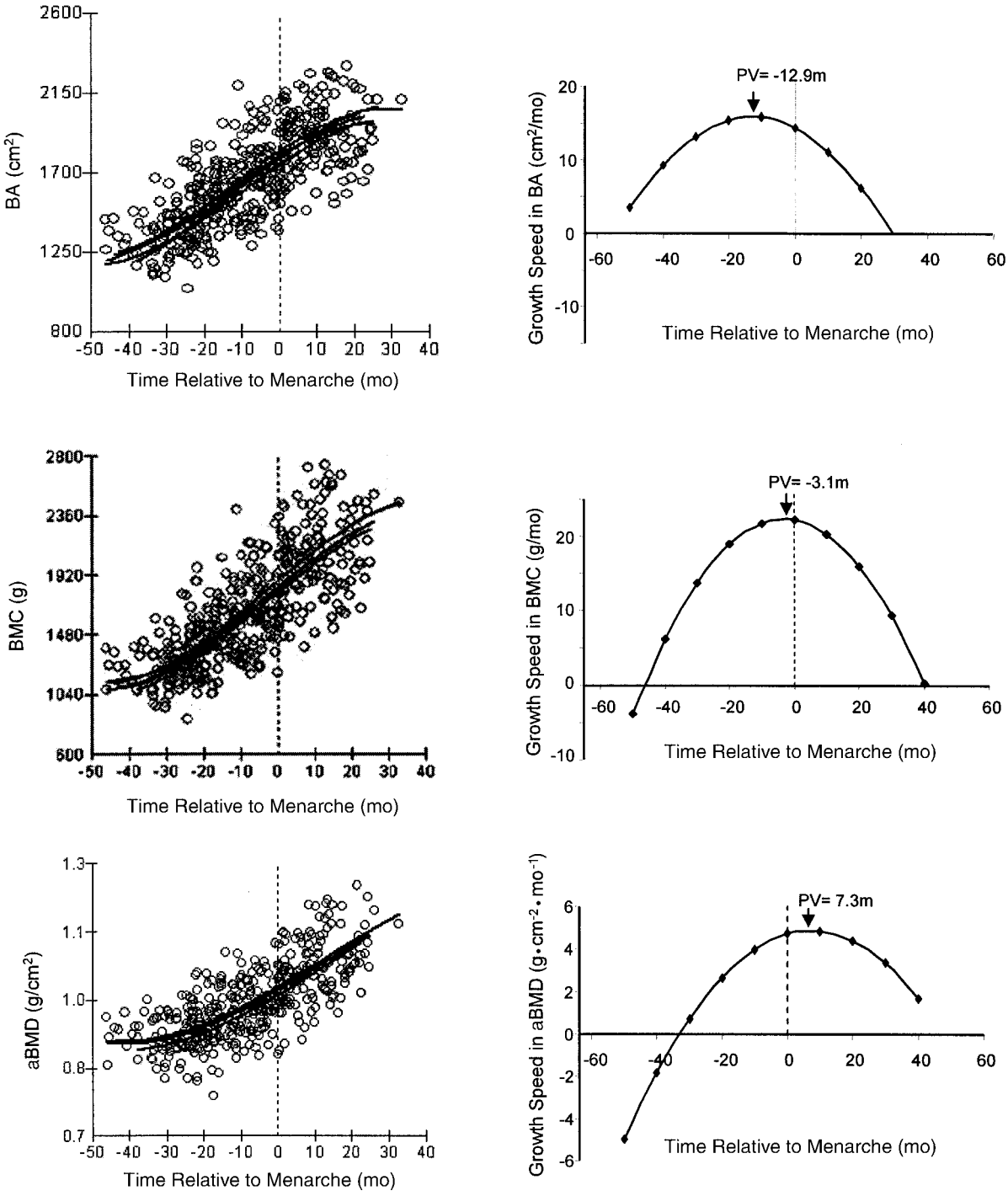

FIGURE 5. Growth curves of the whole-body bone area (BA), bone mineral content (BMC), and bone mineral density (BMD). In the left panel, each circle indicates an individual value. The lines represent the hierarchical linear model with random effects for different groups. Because no group differences were found, the growth speed curves in the right panel are for the total sample. The best-fitting lines were as follows: BA (cm^2) = 1789 + 14.39 × t − 0.116 × t^2 −0.003 × t^3; BMC (g) = 1836 + 22.83 × t − 0.038 × t^2 −0.004 × t^3; and BMD (mg/cm^2) = 1022 + 4.71 × t − 0.022 × t^2 − 0.001 × t^3, where t is time in mo.

distribution of calcium intake during the whole day in the form of food (lower amounts evenly distributed versus larger amounts twice a day), which may also account for the better absorption (11); and *3*) higher intakes of protein, magnesium, or some other micronutrients from dairy products than from pills (12). All groups were similar with respect to nutrient intake, hormone concentrations, and growth variables, which suggests that distributing calcium over the course of the day may be more beneficial.

Like our study, others have shown positive effects of increased calcium intake from the consumption of dairy products on BMD of the spine, hip, and forearm, with varied statistical results (13,

14). However, an effect of higher calcium intake from dairy products appears to be most prominent and statistically significant in indexes of cortical thickness of long bones (13) and in girls whose longitudinal growth had ceased (14), which suggests the importance of adequate dairy consumption during the secondary consolidation process. Ideally, promoting an increase in calcium intake through cheese or other dairy products is the prudent approach. Although the feasibility of success may be questioned in other cultures, the Finnish population has readily embraced recommendations from the government in other areas. The challenge for Finland is keeping children from abandoning their traditional dietary habits while the availability of convenience and fast-food restaurants increases (15).

Dietary intake of vitamin D may be of secondary importance when exposure to ultraviolet rays is optimal. In Finland, the cutaneous synthesis of vitamin D is limited for 9 mo of the year, making the low dietary intake of our population (15) a target for intervention. During the trial, only the Ca+D group exceeded the recommended vitamin D intake and showed increases in their serum concentrations; however, we did not find noticeable effects on bone accrual. Vitamin D supplementation has been shown to uniformly increase serum concentrations when the dose is tied to body weight and is dependent on initial serum vitamin D concentrations in prepubertal children (16). Whether a uniform dose of 200 vitamin IU D_3/d can make a difference in calcium absorption when there is large variability in serum 25(OH)D concentrations and the intake of calcium is high during puberty is not known. It may be that the girls with the lowest 25(OH)D concentrations had the best response, but our study was not designed to answer this question.

Scientific interest in the rapid growth period has focused on the maximization of bone growth to achieve optimal peak bone mass (17–25). The idea is widely accepted that a greater reserve of bone during this period will attenuate the consequences of inevitable bone loss in old age. Through childhood and adolescence, the requirements of normal retention of calcium in bone are estimated, on the basis of balance data or cohort studies, to determine the lowest intake compatible with meeting the accrual needs of adolescents. Due to the inability to identify in which phase of bone growth a child is at a particular time, the requirements established for peak accrual are applied across a wide age range (26, 27). Calcium retention of 300 mg/d is expected during the peak rate of growth when calcium intake is >1000 mg/d (21, 22, 28). During the highest need for calcium during puberty, the absorption and utilization of calcium may also increase, especially at the lowest intake level. Recent data from Abrams et al (29) suggest that dietary intake <400 mg/d has a detrimental effect on calcium balance. The exact calcium intake that results in a negative calcium balance remains to be established experimentally. However, longitudinal studies of adolescents (20, 25, 30) have provided support for moderate levels of calcium having little effect on long-term bone gain. In the present study, calcium intake exceeded the recommended amount in all intervention groups. The placebo group also increased their calcium intakes from 664 to 926 mg/d, and only one child had an intake <400 mg/d throughout the study period. Consequently, almost all the girls could be considered to have relatively "adequate" calcium intakes; therefore, it is hard to tease out difference in the effects of the supplementation between the groups.

The most compelling evidence that calcium consumption influences the rates of bone mineral accrual comes from a few controlled calcium supplementation intervention studies of 1–7 y durations with various doses (300–1200 mg/d) and forms of calcium and in different age groups of children (31–33). Some studies have been relatively short in duration (32, 33) and they indicate that the differential response to calcium supplementation is due to differential growth patterns of different skeletal sites (34), timing of the intervention in relation to menarche (35), and habitual calcium intake (31). Our study provides support for the importance of timing of the intervention in relation to the natural history of bone accrual. Matkovic et al (31) found that calcium supplementation and dairy products had a positive influence on bone mineral density of the hip and the forearm in adolescent females (15–18 y old) after 7 y of intervention and that the effect is dependent on compliance and body frame. Our peak velocity data of aBMD agree with the importance of calcium intake to maximize the secondary consolidation process (20, 30, 36) after menarche. Therefore, as shown by other groups (20, 31), only long-term follow-up studies can provide information on the effect of calcium supplementation on peak bone mass. However, the recent study by Dodiuk-Gad (37) suggests that calcium supplementation during secondary consolidation is retained for up to 1 y after supplementation is complete.

A perplexing problem in Finland, as in many other Nordic countries, is that the proportion of the population with very low calcium intakes is very small (38), yet the fracture rate is high (39). In our case, only 1% of the girls in the total screened population had a dietary calcium intake <400 mg/d. Therefore, a factor other than calcium intake must be playing a major role in defining bone strength and health. On the other hand, this does not mean that adequate calcium intake is not necessary. Calcium supplementation may be useful for those with very low intakes, such as individuals who consume <400 mg/d because of milk allergy or lactose intolerance. However, comprehensive studies need to be conducted with control groups with true calcium-deficient diets, as well as with vitamin D–supplemented groups with low calcium intakes and low vitamin D concentrations, to find out whether calcium or vitamin D supplementation will have beneficial effects in subgroups of growing subjects with truly deficient status and who are at real risk of attaining suboptimal peak bone mass and thus, later, osteoporosis. An important question raised in the present study is how we can identify those at-risk subjects and focus our resources on optimizing their nutritional behavior while avoiding unnecessary supplementation in normal growing children.

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design, biomarkers analysis, and preparation of the article. PHFN was involved in the conception and preparation of the article. KKI was involved in the biomarker analyses and preparation of the article. RK was involved in the funding, conception, study design, and preparation of the article. CO was involved in the conception, funding, and preparation of the article. KHV was involved in the design and funding of the study and preparation of the article. HK was responsible for the medical examination and was involved in the management, conception, design, and funding of the study as well as the preparation of the article. FT was involved in the conception, management, and design of the study; data analysis; and preparation of the article. None of the authors had financial or personal interests or affiliations with the sponsors of this research effort.

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