Bone mineral contents and plasma osteocalcin concentrations of Gambian children 12 and 24 mo after the withdrawal of a calcium supplement[1,2]

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ABSTRACT

Background: Our randomized, placebo-controlled supplementation study of 160 rural Gambian children aged 8.3–11.9 y showed that an increase in calcium intake of 714 mg/d for 12 mo resulted in a 5% increase in forearm bone mineral acquisition and a 22% decrease in plasma osteocalcin concentration, a bone formation marker, but had no effect on height or bone dimensions.

Objective: We investigated whether these results were sustained after supplement withdrawal.

Design: All participants were followed up 12 (FU1) and 24 (FU2) mo after supplementation ended. Bone mineral content (BMC), bone mineral density (BMD), and BMC adjusted for bone width, body weight, and height (size-adjusted BMC) were measured at the midshaft and distal radius. Plasma osteocalcin concentration was measured at FU1.

Results: At follow-up, the calcium group had greater bone mineral status than did the placebo group at the midshaft radius (mean difference ± SE), FU1: BMC (4.7 ± 1.6%; $P = 0.004$), BMD (5.1 ± 1.1%; $P \leq 0.0001$), size-adjusted BMC (5.0 ± 1.1%; $P \leq 0.0001$); FU2: BMC (3.8 ± 1.6%; $P = 0.02$), BMD (2.7 ± 1.3%; $P = 0.04$), size-adjusted BMC (2.5 ± 1.3%; $P = 0.06$). Similar differentials were observed at the distal radius but were not significant. No significant differences in plasma osteocalcin concentrations (FU1: −0.5 ± 6.5%; $P = 0.9$) were observed between groups.

Conclusion: Although some of the effects of calcium supplementation were still evident at follow-up, further studies are required to determine whether short-term increases in calcium intake have lasting benefits for Gambian children. *Am J Clin Nutr* 2002;76:681–6.

KEY WORDS Bone mineral accretion, calcium, children, Gambia, osteocalcin, osteoporosis

INTRODUCTION

Osteoporosis is a skeletal disease characterized by low bone mass and deterioration of bone tissue, resulting in an increased incidence of fragility fractures (1). Bone mass in young adulthood is a major predictor of later fracture risk (2, 3). Understanding the factors that influence bone mass accumulation in childhood and maximize peak bone mass in young adulthood, therefore, is important for designing preventative strategies to combat osteoporosis (4).

Currently, considerable interest is focused on the importance of calcium nutrition in childhood and the development of peak bone mass. Several studies have shown a significant effect of a calcium supplement or milk on bone mineral acquisition (5–12). However, to date, only a few studies have made follow-up measurements after the supplement was withdrawn to investigate long-lasting benefits. Of these, most studies failed to show a sustained effect of calcium supplementation following supplement withdrawal (13–15). An exception was a study of prepubertal girls who consumed foods fortified with a calcium supplement derived from milk: a sustained effect on bone mineral acquisition after the end of the supplementation period was documented (9).

In our recent calcium supplementation study of Gambian children accustomed to a low calcium intake of ≈300 mg (7.5 mmol)/d, we showed that an increase in calcium intake of 714 mg (17.85 mmol)/d for 12 mo resulted in a greater bone mineral content at the midshaft radius and distal radius by ≈5% with no effect on height or bone dimensions (12). This was accompanied by a 22% decrease in plasma osteocalcin concentration, a bone formation marker. The present study examined whether the increase in bone mineral status of the Gambian children associated with the calcium supplement was sustained 12 and 24 mo after supplement withdrawal.

SUBJECTS AND METHODS

One hundred sixty healthy Gambian children (80 boys and 80 girls) who had previously taken part in a randomized, double-blind supplementation study in which they consumed either a calcium supplement or a matching placebo for 12 mo were invited to visit the clinic at MRC Keneba for follow-up measurements 12 (FU1) and 24 (FU2) mo after supplementation had stopped. The children were from the rural village of Keneba,

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West Kiang, and were aged 8.3–11.99 y at the start of the supplementation study (baseline). All 160 children who were measured at baseline participated in the follow-up studies at FU1 and FU2.

The calcium supplement was 1000 mg (25 mmol) chewable calcium carbonate (Calcichew; Shire Pharmaceuticals Ltd, Andover, United Kingdom, and Nycomed Pharma AS, Oslo), consumed 5 d/wk to provide an average calcium intake of 714 mg/d (17.85 mmol/d) over the year of the study. The placebo tablets, produced by the same manufacturers, were of similar shape, taste, and texture. A full description of the study population and the calcium supplementation study was given previously (12). The subjects and investigators involved in data collection in The Gambia and Cambridge remained blind to the tablet assignments throughout the supplementation and follow-up periods.

Follow-up measurements were made 12 and 24 mo after supplement withdrawal ($\bar{x} \pm$ SD: FU1, 367 ± 29 d; FU2, 732 ± 30 d). There was no significant difference in the timing of these measurements between the calcium and placebo groups (FU1: 364 ± 2 and 369 ± 41 d in the calcium and placebo groups, respectively; FU2: 731 ± 10 and 733 ± 41 d in the calcium and placebo groups, respectively). The follow-up measurements for each individual took place 24 and 36 mo after his or her original baseline measurements before supplementation (FU1, 752 ± 27 d; FU2, 1117 ± 29 d). No subject had a history of any medical condition known to affect calcium and bone metabolism or had a recent fracture. None were consumers of alcohol, antacids, calcium, or other nutritional supplements; none were smokers; and none of the girls were taking contraceptive pills.

The study was approved by the MRC/Gambian Government Ethics Committee. The follow-up measurements were an integral part of the study protocol, and informed consent was obtained from the children and their parents at enrollment.

Bone mineral status

Measurements of bone mineral content (BMC; g/cm), bone width (BW; cm), and bone mineral density (BMD; g/cm^2) at the midshaft and distal radius of the left arm were made with the use of single-photon absorptiometry (Lunar SP2; Lunar Radiation Corporation, Madison, WI). Full details of the technique used in this study were described previously (12).

The instrument was stable throughout the 3 y of the supplementation and follow-up studies. Coefficients of variation for BMC, BW, and BMD of phantoms provided by the manufacturer were 1.13%, 0.85%, and 0.74% for the small phantom (BMC = 0.374 g/cm) and 0.56%, 0.40%, and 0.62% for the large phantom (BMC = 1.196 g/cm), with no sign of drift. The in vivo precision of the instrument, estimated from replicate measurements of children with repositioning, was 1.1% for BMC and 2.5% for BW (16).

Anthropometry and pubertal status

Each subject was weighed to the nearest 0.1 kg while wearing light clothing and no shoes. Standing height was measured to the nearest 0.1 cm in subjects not wearing shoes. Sexual maturity was assessed according to Tanner stages of classification (17). Pubertal status of the girls was based on breast and pubic hair development and was assessed by a female pediatric physician or the senior midwife at the MRC Keneba Clinic. Assessment of the sexual maturity of the boys was based on genital and pubic hair development and was conducted at FU1 by the principal investigator (B Dibba) and at FU2 by a male pediatric physician.

Plasma osteocalcin

Blood was obtained for osteocalcin analysis at FU1 from a subset of 100 subjects (equally divided between the calcium and placebo groups) for whom plasma osteocalcin values were available both at baseline and after 12 mo of supplementation. Each sample was collected between 0630 and 0700 after the subjects had fasted overnight. The blood was anticoagulated with lithium heparin, kept cool, and centrifuged at 1700 × g for 20 min at 4 °C within 45 min. The plasma was separated and stored frozen. The samples were transported on dry ice to Cambridge. Plasma intact osteocalcin was quantified with the use of an immunoradiometric assay (N-TACT Osteocalcin; INCStar Corporation, Stillwater, MN), which had a between-run precision of 6% during the study period for a control sample with a concentration of 26 μg/L, measured in duplicate.

Dietary calcium intake

Dietary calcium intake was quantified at FU1 by direct weighing of all foods consumed for 2 d (12). Computation of nutrient intakes was carried out with the use of the Gambian Dido and MW1N4 in-house suite of software programs based on McCance and Widdowson's food-composition data, supplemented by information on the composition of Gambian foods (18). Drinking water in Keneba has a low calcium concentration (< 10 mg/L) (18), and its consumption was not quantified.

Statistical analysis

Descriptive statistics are presented as means ± SDs and differences ± SEs. Statistical analysis was performed by using simultaneous multiple regression analysis, analysis of variance, and analysis of covariance with Scheffe's post hoc tests (LINEAR MODEL SOFTWARE, DATADESK 4.1; Data Description Inc, Ithaca, NY). Baseline value was included as an independent variable in all models to minimize the effects of regression toward the mean. All continuous variables, except age, were converted to natural logarithms to facilitate examination of power relationships between continuous variables and to investigate proportional effects of discrete variables (19, 20). The regression coefficient for a discrete variable, when the dependent variable is in natural logarithms, once multiplied by 100, corresponds closely to the percentage effect as defined by (difference/mean) × 100 (21). In all cases the distribution of the log-transformed variables approximated normality.

The effect of the calcium supplement at FU1 and FU2 was assessed as the percentage difference between treatment groups at each time point, after correcting for baseline value and potential confounding variables. The result is equivalent to the difference between the treatment groups in percentage change since baseline, ie, over the full 24- and 36-mo period, respectively, after adjusting for baseline value. The effect of the supplement on BMC independent of bone and body size was examined at each time point by including the mean and difference since baseline for BW, weight, and height as independent variables in the regression analyses. Other independent variables examined were age, sex, pubertal status, calcium intake, and interaction terms as appropriate. In each case a full regression model was constructed, which included all relevant independent variables, followed by the removal of nonsignificant variables by backward elimination. Similar models were constructed to examine the effect of the supplement on height, bone width, and plasma osteocalcin concentration. The approach to the statistical analysis and full details of the modeling procedure were described previously (12, 19).

TABLE 1
Characteristics and anthropometric measures of the calcium-supplemented and placebo groups[1]

	Baseline		FU1		FU2	
	Calcium group	Placebo group	Calcium group	Placebo group	Calcium group	Placebo group
Age (y)[2]	10.3 ± 1.0[3]	10.3 ± 1.0	12.3 ± 1.0	12.3 ± 1.0	13.3 ± 1.0	13.3 ± 1.0
Weight (kg)[2]	25.5 ± 4.0	24.9 ± 4.1	30.9 ± 6.3	29.9 ± 5.6	34.6 ± 7.5	33.1 ± 6.7
Height (cm)[2]	132.5 ± 6.9	131.6 ± 7.6	142.7 ± 7.7	141.6 ± 8.3	147.4 ± 7.8	146.3 ± 8.7
MUAC (cm)[2]	18.2 ± 1.6	18.0 ± 1.6	20.1 ± 2.1	19.7 ± 1.9	21.3 ± 2.9	20.6 ± 2.2
Triceps skinfold thickness (mm)[2]	8.0 ± 1.7	7.9 ± 2.1	8.4 ± 2.7	8.3 ± 2.9	8.7 ± 3.4	8.3 ± 2.8
Grip strength (kg)[2]	11.1 ± 3.0	10.7 ± 2.7	17.1 ± 4.0	16.3 ± 3.1	19.4 ± 5.0	18.2 ± 4.6
Calcium intake (mg/d)	342 ± 129	334 ±153	360 ± 164	343 ± 116	—	—
Tanner stage ≥ 3 [n (%)]						
Boys[4]	0 (0)	0 (0)	5 (12.5)	8 (20.0)	9 (22.5)	7 (17.5)
Girls[4]	3 (7.5)	1 (2.5)	11 (27.5)	9 (22.5)	16 (40.0)	15 (37.5)

[1] n = 80/group. Baseline measurements were taken before the 12-mo calcium supplementation study. FU1, follow-up 12 mo after supplement withdrawal; FU2, follow-up 24 mo after supplement withdrawal; MUAC, midupper arm circumference.

[2] Each variable was significantly greater at FU2 than at FU1 and at FU1 than at baseline, $P < 0.0001$ (two-factor repeated-measures ANOVA with Scheffe's post hoc test), except for the difference between FU1 and baseline for MUAC and triceps skinfold thickness (not significant). There were no significant main effects of group or significant interactions between group and time for any variable.

[3] $\bar{x}$ ± SD.

[4] n = 40/group.

Subject characteristics at baseline and the response to calcium supplementation were not different in boys and girls (12). Similar findings, of similar magnitude, were observed when analyzing the follow-up data for each sex separately, and no significant sex-by-supplement group interactions were obtained in the regression analyses. Consequently, the follow-up data in this report are presented with boys and girls together.

RESULTS

The characteristics of the calcium and placebo groups before supplementation and at FU1 and FU2 are given in **Table 1**. The children at follow-up were small for their age and puberty was delayed (17, 22). The mean ± SD z scores relative to the British reference were height-for-age z score: FU1, –1.26 ± 0.90; FU2, –1.47 ± 0.94; weight-for-age z score: FU1, –1.92 ± 0.92; FU2, –1.85 ± 1.01. There was no significant difference between the calcium and placebo groups in the pubertal-stage profile of either the boys or girls. At baseline, 85% boys and 76% of girls were prepubertal, and by FU2, 68% of the boys and 21% of the girls remained in Tanner stage 1. The proportions of subjects who had progressed to Tanner stage ≥3 at FU1 and FU2 are given in Table 1. The numbers of girls who had experienced menarche by FU1 and FU2 were 3 (4%) and 8 (10%), respectively. Dietary calcium intake at FU1 was not different to that at baseline (Table 1), and there was no difference between the groups in the intake of calcium (Table 1) or of energy, protein, fat, or phosphorus (data not shown).

By FU1 and FU2 both groups of children were significantly heavier and taller than at baseline and had greater BMC, BW, and BMD at the midshaft and distal radius (Table 1 and **Table 2**). The increases in weight and height and in bone variables at the midshaft radius were greater in girls than in boys and in those who had entered puberty than in those who had not, but were generally independent of age (**Tables 3–5**). The effects of pubertal stage on midshaft BMC disappeared after size adjustment.

At the midshaft radius, the incremental gains since baseline of BMC, BMD, and size-adjusted BMC were significantly greater in the calcium group than in the placebo group (Tables 3 and 4). Findings of similar magnitude were observed at the distal radius, but the differences between the groups were not statistically significant (Tables 3 and 4). The magnitudes of the differences and, for the midshaft radius the strengths of the effects, at FU1 were not different from those observed between the calcium and placebo groups at the end of the supplementation period, but were lower and less significant at FU2 (12). The effects of the calcium supplement on bone mineral status were not influenced by adjusting for age, sex, pubertal status, or dietary calcium intake, and no interactions were observed. There were no significant effects of the calcium supplement at either FU1 or FU2 on incremental gain in body weight, height, or BW at the midshaft or distal radius.

Plasma osteocalcin concentrations were higher at FU1 than at baseline ($P < 0.001$, Table 2). This increase was greater in older children and in girls than in boys but was independent of pubertal status (Table 5). There was no significant difference in plasma osteocalcin concentration at FU1 between the calcium and placebo groups, before or after adjustment for baseline value, age, pubertal status, and sex (Table 5).

DISCUSSION

This study showed that the effect of calcium supplementation on bone mineral status was sustained 12 mo after supplement withdrawal and that some residual effects were still evident after 24 mo. At FU1, the magnitude of the effect on bone mineral status at the midshaft radius, expressed as BMC, BMD, or size-adjusted BMC, was commensurate with that seen at the end of the supplementation period. By FU2, the difference between the groups at the midshaft radius was attenuated but was still statistically significant. The effect at the distal radius was of a similar magnitude but was not statistically significant, possibly reflecting the greater imprecision of measurements at this site. Conversely, no difference in plasma osteocalcin concentration remained between the calcium and placebo groups at FU1, in spite of the large difference observed at the end of the supplementation period. No effect of the calcium supplement on bone and body size or on pubertal status was noted at any time.

These results suggest a possible long-term effect of calcium supplementation on BMC in Gambian children, with no effect on

TABLE 2
Bone measures and plasma osteocalcin concentrations in the calcium-supplemented and placebo groups[1]

	Baseline		FU1		FU2	
	Calcium group	Placebo group	Calcium group	Placebo group	Calcium group	Placebo group
Midshaft radius[2]						
BMC (g/cm)[3]	0.452 ± 0.087	0.434 ± 0.085	0.539 ± 0.095	0.502 ± 0.100	0.584 ± 0.103	0.549 ± 0.115
BW (cm)	0.976 ± 0.117	0.960 ± 0.114	1.034 ± 0.114	1.025 ± 0.117	1.090 ± 0.120	1.064 ± 0.122
BMD(g/cm^2)[3]	0.461 ± 0.058	0.451 ± 0.062	0.520 ± 0.053	0.488 ± 0.066	0.534 ± 0.058	0.513 ± 0.073
Distal radius[2]						
BMC (g/cm)	0.419 ± 0.096	0.388 ± 0.103	0.519 ± 0.121	0.478 ± 0.124	0.580 ± 0.211	0.516 ± 0.122
BW (cm)	1.828 ± 0.183	1.777 ± 0.198	2.017 ± 0.207	1.963 ± 0.220	2.077 ± 0.276	2.016 ± 0.205
BMD (g/cm^2)	0.227 ± 0.040	0.217 ± 0.044	0.256 ± 0.043	0.242 ± 0.048	0.273 ± 0.070	0.254 ± 0.045
Osteocalcin (μg/L)[4]	24.3 ± 10.5	23.5 ± 7.9	32.1 ± 14.5	31.8 ± 12.8	—	—

[1] $\bar{x}$ ± SD; n = 80/group. Baseline measurements were taken before the 12-mo calcium supplementation study. FU1, follow-up 12 mo after supplement withdrawal; FU2, follow-up 24 mo after supplement withdrawal; BMC, bone mineral content; BW, bone width; BMD, bone mineral density.

[2] Each variable was significantly greater at FU2 than at FU1 and at FU1 than at baseline, $P < 0.001$ (two-factor repeated-measures ANOVA with Scheffe's post hoc test). There were no significant differences between the groups for any variable at baseline.

[3] There was a significant interaction between intervention group and time for midshaft BMC ($P = 0.046$) and BMD ($P = 0.002$) but for no other variable, $P \leq 0.05$ (two-factor repeated-measures ANOVA with interaction).

[4] n = 50/group.

growth. This differs from the results of 3 studies in which the effect of supplementation with calcium salts, including calcium carbonate as used in the Gambian study, was not sustained after supplement withdrawal (13–15). A continuing effect 1 y after supplementation with foods fortified with calcium phosphate, extracted from milk, was reported from one study of well-nourished prepubertal girls, but, unlike the Gambian study, this appeared to be associated with differences in growth velocity (9).

It has been hypothesized that the increase in BMC with no concomitant increase in bone size that is observed when children are supplemented with calcium salts may be the result of a phenomenon known as the bone remodeling transient, in which the increase in calcium supply causes a decrease in osteoclast activation frequency and a subsequent decrease in the volume of bone undergoing remodeling at any one time (12, 23, 24). If this were the case, the increase in bone mineral would be expected to be temporary and to reach a plateau when the bone turnover rate attained a new steady state after all resorption cavities excavated at the previous activation frequency had been remodeled. It would also be predicted that the differential in bone mineral

TABLE 3
Effect of calcium supplementation and other variables on bone measures at follow-up 12 mo after supplement withdrawal (FU1)[1]

	BMC	BW	BMD	Size-adjusted BMC
		% (P)		
Midshaft radius				
Supplement group	4.7 ± 1.6[2] (0.004)	−0.3 ± 0.8 (0.7)	5.1 ± 1.1 (≤0.0001)	5.0 ± 1.1 (≤0.0001)
Baseline value	0.8 ± 0.04 (≤0.0001)	0.8 ± 0.04 (≤0.0001)	0.7 ± 0.04 (0.001)	0.7 ± 0.04 (≤0.0001)
Sex	3.7 ± 1.6 (0.02)	NS	3.5 ± 1.1 (0.002)	3.5 ± 1.1 (0.001)
Pubertal stage	6.7 ± 2.1 (0.002)	3.3 ± 1.1 (0.003)	3.4 ± 1.5 (0.02)	NS
Change in BW	—	—	—	1.2 ± 0.1 (≤0.0001)
Mean BW	—	—	—	0.3 ± 0.1 (≤0.0001)
Mean weight	—	—	—	0.1 ± 0.04 (0.006)
Constant	−9.3 ± 3.9 (0.02)	5.0 ± 0.7 (≤0.0001)	−17.9 ± 3.6 (≤0.0001)	−62 ± 16 (0.0001)
Distal radius				
Supplement group	4.5 ± 3.0 (0.1)	0.9 ± 1.3 (0.5)	4.2 ± 2.5 (0.09)	2.7 ± 2.4 (0.3)
Baseline value	0.5 ± 0.06 (≤0.0001)	0.6 ± 0.06 (≤0.0001)	0.4 ± 0.07 (≤0.0001)	0.4 ± 0.07 (≤0.0001)
Sex	NS	NS	NS	NS
Pubertal stage	8.3 ± 4.1 (0.04)	NS	7.2 ± 3.3 (0.03)	NS
Change in BW	—	—	—	0.8 ± 0.1 (≤0.0001)
Mean BW	—	—	—	1.1 ± 0.2 (≤0.0001)
Mean weight	—	—	—	NS
Constant	−26 ± 7 (0.0002)	30 ± 4 (≤0.0001)	−79 ± 11 (≤0.0001)	—118 ± 16 (≤0.0001)

[1] BMC, bone mineral content; BW, bone width; BMD, bone mineral density.

[2] Mean coefficients ± SE from simultaneous multiple regression analysis with the value at FU1 as the dependent variable. All continuous variables except age were in natural logarithms and multiplied by 100. The results for discrete variables correspond to the percentage difference in the dependent variable between groups (*see* Subjects and Methods). Full models were set up with supplement group (calcium group = 1, placebo group = 0), baseline value, sex (F = 1, M = 0), age at baseline, and pubertal status at baseline (Tanner stage ≥2 = 1, Tanner stage 1 = 0) as independent variables. Nonsignificant variables other than supplement group were removed by backward elimination ($P > 0.05$) to produce the parsimonious models presented. For size-adjusted BMC, change and mean of the log-transformed values for BW, weight, and height were also included (*see* Subjects and Methods). Age, change in weight, change in height, and mean height were not significant in any model at FU1 and are not shown.

TABLE 4
Effect of calcium supplementation on bone measures at follow-up 24 mo after supplement withdrawal (FU2)[1]

	BMC	BW	BMD	Size-adjusted BMC
		% (P)		
Midshaft radius				
Supplement group	3.8 ± 1.6[2] (0.02)	1.2 ± 0.9 (0.2)	2.7 ± 1.3 (0.04)	2.5 ± 1.3 (0.06)
Baseline value	0.8 ± 0.04 (≤0.0001)	0.8 ± 0.04 (≤0.0001)	0.7 ± 0.05 (≤0.0001)	0.7 ± 0.05 (≤0.0001)
Sex	7.6 ± 1.6 (≤0.0001)	2.5 ± 0.9 (0.01)	5.1 ± 1.3 (0.0002)	5.2 ± 1.4 (0.0002)
Pubertal stage	6.6 ± 2.2 (0.003)	3.2 ± 1.3 (0.01)	3.8 ± 1.7 (0.03)	NS
Change in BW	—	—	—	0.9 ± 0.1 (≤0.0001)
Mean BW	—	—	—	0.3 ± 0.1 (0.001)
Mean weight	—	—	—	0.1 ± 0.05 (0.03)
Constant	−1.2 ± 3.9 (0.8)	7.5 ± 0.9 (≤0.0001)	−13 ± 4 (0.002)	−52 ± 19 (0.006)
Distal radius				
Supplement group	1.5 ± 3.4 (0.7)	0.3 ± 1.5 (0.8)	2.4 ± 2.6 (0.4)	1.8 ± 2.4 (0.5)
Baseline value	0.8 ± 0.07 (≤0.0001)	0.8 ± 0.07 (≤0.0001)	0.7 ± 0.07 (≤0.0001)	0.5 ± 0.07 (≤0.0001)
Sex	NS	NS	NS	NS
Pubertal stage	NS	NS	NS	NS
Change in BW	—	—	—	1.3 ± 0.1 (≤0.0001)
Mean BW	—	—	—	1.0 ± 0.3 (≤0.0001)
Mean weight	—	—	—	NS
Constant	13 ± 7 (0.07)	26 ± 4 (≤0.0001)	−29 ± 11 (0.006)	−97 ± 17 (≤0.0001)

[1] BMC, bone mineral content; BW, bone width; BMD, bone mineral density.

[2] Mean coefficients ± SE from simultaneous multiple regression analysis with the value at FU2 as the dependent variable. All continuous variables except age were in natural logarithms and multiplied by 100. The results for discrete variables correspond to the percentage difference in the dependent variable between groups (*see* Subjects and Methods). Full models were set up with supplement group (calcium group = 1, placebo group = 0), baseline value, sex (F = 1, M = 0), age at baseline, and pubertal status at baseline (Tanner stage ≥2 = 1, Tanner stage 1 = 0) as independent variables. Nonsignificant variables other than supplement group were removed by backward elimination ($P > 0.05$) to produce the parsimonious models presented. For size-adjusted BMC, change and mean of the log-transformed values for BW, weight, and height were also included (*see* Subjects and Methods). Age, change in weight, change in height, and mean height were not significant in any model at FU2 and are not shown.

would disappear when the rate of bone turnover returned to its former level after calcium supplementation was withdrawn.

The findings of the Gambian study do not fit well with this simple model. Differences in plasma osteocalcin concentration had disappeared 12 mo after supplement withdrawal, but the effects on BMC in the forearm had not and were still evident after 24 mo. One interpretation is that bone remodeling in Gambian children may be slower than in other populations, so that the reversal of the bone remodeling transient took many more months to complete than was noted in other studies. This possibility is supported by the finding that American black children have lower bone remodeling rates than do white children of the same age, as shown by plasma osteocalcin concentration and tartrate-resistant acid phosphatase activity (15). However, data on this point are not consistent, because no differences were noted in urinary bone resorption markers between American black and white children (25). Also the use of bone turnover markers in children has been shown to be problematic because they may underestimate true bone turnover (26). However, should this interpretation prove correct it would imply that short-term calcium supplementation does not result in a long-lived alteration in bone mineral status.

TABLE 5
Effect of calcium supplementation on height, weight, and plasma osteocalcin concentrations at follow-up

	Height		Weight		Osteocalcin
	FU1	FU2	FU1	FU2	FU1
			% (P)		
Supplement group	0.1 ± 0.3[2] (0.8)	0.0 ± 0.3 (0.9)	0.3 ± 0.9 (0.8)	1.7 ± 1.2 (0.1)	−0.5 ± 6.5 (0.9)
Baseline value	0.9 ± 0.03 (≤0.0001)	1.0 ± 0.03 (≤0.0001)	1.1 ± 0.03 (≤0.0001)	1.1 ± 0.05 (≤0.0001)	0.4 ± 0.08 (≤0.0001)
Sex	1.1 ± 0.3 (0.0002)	1.7 ± 0.3 (≤0.0001)	4.7 ± 0.9 (≤0.0001)	9.6 ± 1.2 (≤0.0001)	18 ± 7 (0.009)
Pubertal stage	0.9 ± 0.4 (0.02)	NS	4.0 ± 1.4 (0.004)	NS	NS
Age	NS	NS	NS	2.0 ± 0.8 (0.01)	12.7 ± 5.1 (0.01)
Constant	38 ± 14 (0.008)	33 ± 15 (0.03)	−4.8 ± 11 (0.7)	−22 ± 12 (0.07)	58 ± 53 (0.3)

[1] FU1, follow-up 12 mo after supplement withdrawal; FU2, follow-up 24 mo after supplement withdrawal.

[2] Mean coefficients ± SE from simultaneous multiple regression analysis with the value at FU1 or FU2 as dependent variable. All continuous variables except age were in natural logarithms and multiplied by 100. The results for discrete variables correspond to the percentage difference in the dependent variable between groups (*see* Subjects and Methods). Full models were set up with supplement group (calcium group = 1, placebo group = 0), baseline value, sex (F = 1, M = 0), age at baseline, and pubertal status at baseline (Tanner stage ≥2 = 1, Tanner stage 1 = 0) as independent variables. Nonsignificant variables other than supplement group were removed by backward elimination ($P > 0.05$) to produce the parsimonious models presented.

Another plausible interpretation is that bone resorption remained at a lower rate in the calcium than in the placebo group at follow-up but that the circulating concentration of osteocalcin, produced by osteoblasts during bone formation, does not adequately reflect osteoclast activation frequency in growing children, despite its use as a marker of bone turnover in adults. A similar dissociation of effects of a calcium intervention on bone formation and bone resorption markers was noted in white children (26). If this were the case, it would imply that short-term calcium supplementation in children results in a reprogramming of bone remodeling, producing a long-term effect on bone mineral status. Further, the lower bone remodeling rate might prove to be beneficial, because lower rates of bone remodeling have been associated with higher peak bone mass (15), suggesting that the rate of bone turnover during childhood and early adulthood may be an important factor underlying future fracture risk. Clearly, a more detailed profile of calciotropic hormone concentrations and bone turnover markers is needed to more fully explore the mechanism underlying these results.

Finally, the results of this study may support the possibility that, because in children bone formation exceeds bone resorption, calcium supplementation results in a more positive remodeling balance (23). Although some of the gain in accreted bone might be expected to be lost on supplement withdrawal because of the remodeling transient, a more positive remodeling balance would be predicted to produce permanent gains in bone accretion (23). That previous studies have not shown residual effects of calcium supplementation in children may be due to differences in sample size at follow-up, calcium dosage, compliance rates, or inherent differences between populations. Further follow-up studies of the Gambian cohort are required to investigate this further.

In summary, this study showed that the increase in bone mineral status that resulted from the consumption by rural Gambian children of an extra 714 mg Ca/d for 12 mo was sustained 12 and 24 mo after the withdrawal of the calcium supplement. The gain in bone mineral, if maintained into adulthood, would increase peak bone mass and might lead to a reduction in the risk of fractures in later life. However, whether the effect is sustained long enough to maximize the peak bone mass of these children as they progress through puberty and into early adulthood is not known. A further follow-up study is under way to determine whether the short-term increase in calcium intake by Gambian children accustomed to a low calcium intake is sufficient to sustain an increase in bone mass over many years.

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