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Prevention of nutritional rickets in Nigerian children with dietary calcium supplementation ☆

Tom D. Thacher [a,*], Philip R. Fischer [d], Christian O. Isichei [b], Ayuba I. Zoakah [c], John M. Pettifor [e]

[a] Department of Family Medicine (TDT), Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, USA
[b] Department of Chemical Pathology (COI), Department of Community Health (AIZ), Jos University Teaching Hospital, P.M.B. 2076, Jos, Nigeria
[c] Department of Community Health (AIZ), Jos University Teaching Hospital, P.M.B. 2076, Jos, Nigeria
[d] Department of Pediatric and Adolescent Medicine (PRF), Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, USA
[e] MRC Developmental Pathways for Health Research Unit (JMP), Department of Paediatrics, University of the Witwatersrand and Chris Hani Baragwanath Hospital, P O Bertsham 2013, South Africa

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ABSTRACT

Nutritional rickets in Nigerian children usually results from dietary calcium insufficiency. Typical dietary calcium intakes in African children are about 200 mg daily (approximately 20–28% of US RDAs for age). We sought to determine if rickets could be prevented with supplemental calcium or with an indigenous food rich in calcium. We enrolled Nigerian children aged 12 to 18 months from three urban communities. Two communities were assigned calcium, either as calcium carbonate (400 mg) or ground fish (529 ± 109 mg) daily, while children in all three communities received vitamin A (2500 IU) daily as placebo. Serum markers of mineral homeostasis and forearm bone density (pDEXA) were measured and radiographs were obtained at enrollment and after 18 months of supplementation. The overall prevalence of radiographic rickets at baseline was 1.2% and of vitamin D deficiency [serum 25(OH)D < 12 ng/ml] 5.4%. Of 647 children enrolled, 390 completed the 18-month follow-up. Rickets developed in 1, 1, and 2 children assigned to the calcium tablet, ground fish, and control groups, respectively (approximate incidence 6.4/1000 children/year between 1 and 3 years of age). Children who developed rickets in the calcium-supplemented groups had less than 50% adherence. Compared with the group that received no calcium supplementation, the groups that received calcium had a greater increase in areal bone density of the distal and proximal 1/3 radius and ulna over time ($P < 0.04$). We conclude that calcium supplementation increased areal bone density at the radius and ulna, but a larger sample size would be required to determine its effect on the incidence of rickets.

Introduction

Nutritional rickets remains prevalent throughout the world, and in several tropical countries is a consequence of inadequate dietary calcium rather than vitamin D deficiency [1]. Rickets in these children can be effectively treated by calcium supplementation with or without vitamin D [2]. Calcium deficiency occurs in the context of a diet lacking in dairy products. In Nigerian children, the daily dietary intake of calcium is about 200 mg [3], well below the recommended intake of 700–1000 mg for children between one and eight years of age [4]. Though calcium is effective in treating rickets in Nigeria, it is unknown whether calcium supplementation can prevent rickets.

Sustainable, feasible interventions to improve calcium status should be food-based. A study of young children in Bangladesh suggested that rickets could be prevented with a milk-powder-based supplement [5]. Enriching the diet with inexpensive, locally acceptable food sources of calcium may prevent rickets in African children. Dried fish is a common dietary ingredient in Nigeria that could provide calcium if the bones were also consumed [6].

We conducted a controlled clinical trial of calcium supplementation at a community level in urban Nigerian children, enrolled between 12 and 18 months of age. Our primary objective was to test the hypothesis that calcium supplementation, during the age interval of greatest risk for development of rickets, could prevent rickets in Nigerian children. Secondary objectives were to determine if ground fish would be as effective as calcium tablets in preventing rickets, to determine the effect of calcium supplementation on bone mineral acquisition in the forearm, and to assess the effect of calcium supplementation on the calcium-vitamin D axis.

Materials and methods

Subjects

Three geographically separate urban communities (Nassarawa, Gangare, and Dogon Agogo) in the city of Jos, Nigeria (2000 population

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* Corresponding author. Fax: +1 507 284 5067.
E-mail address: thacher.thomas@mayo.edu (T.D. Thacher).

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600,155), where rickets is prevalent, were selected for intervention. We chose communities with similar ethnic, religious, and socioeconomic characteristics and with well-functioning primary health facilities. Each community had one primary health center. Personnel in each center were trained in data collection, assessment of adherence, and conduct of the study, and the quality of data collection was monitored by one of the investigators (TDT).

Children between 12 and 18 months of age who resided in the designated community were eligible for enrollment. This age range represented the usual age of onset of rickets in this population. All eligible children presenting for routine growth monitoring and immunizations over a 5 month period were invited to participate. Information was collected regarding age, ethnic group, father's religion (affects dress, diet, and cultural practices), family history of rickets, age of walking, breast feeding status, usual dairy product intake, and parental occupations and education. Parental occupation was coded with a modified classification ranging from 1 for professional, senior officials to 8 for farm workers [7]. Written informed consent was obtained from a parent or guardian. The Ethical Committee of Jos University Teaching Hospital, the primary health centers, and the Institutional Review Board of Mayo Clinic approved the study. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Data and sample collection

Standing height was measured with wall-mounted stadiometers standardized between the three centers. Length was measured with a lengthboard if the child could not stand. Weight was measured with a hanging weighing scale. Anthropometric z-scores were calculated with Epi Info 3.2.2 (CDC, Atlanta, GA). All children were examined for signs of rickets. Radiographs of the wrists and knees were obtained, and rickets was defined as a radiographic score of 2 or greater on a 10-point scale [8]. Children with rickets at enrollment were excluded and referred for treatment.

Blood was collected by venipuncture, and serum was stored at −20 °C until transported frozen to the Mayo Clinic. Serum calcium, phosphorus, alkaline phosphatase, and albumin were determined by standard methods. Concentrations of 25-hydroxyvitamin D [25(OH) D] and 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] were measured by radioimmunoassay (DiaSorin®, Stillwater, MN).

Dual energy X-ray absorptiometry was performed on the left radius and ulna by a single investigator (TDT) with a portable densitometer (Norland pDEXA, Model 476A110). Measurement sites included the area of minimal bone density of the distal radius and ulna and the proximal 1/3 of the radius and ulna. The instrument was calibrated daily. Duplicate scans in 37 children demonstrated short-term *in vivo* precision of areal bone density of 0.007 g/cm^2 (6.4%) at the distal radius and ulna and 0.013 g/cm^2 (7.2%) at the proximal 1/3 radius and ulna [9]. We used a bone phantom to assess long-term *in vitro* precision of areal bone density, which was 0.11 g/cm^2 (1.1%).

Intervention

Each health center was randomly allocated to dispense vitamin A only (Nassarawa), vitamin A plus calcium tablets (Dogon Agogo), or vitamin A plus ground fish (Gangare). We did not randomize individual subjects in order to simplify logistics and avoid potential contamination of treatment assignments. Vitamin A was chosen as a control supplement, because of its health benefits unrelated to bone [10–12]. Vitamin A was dispensed in prefilled syringes for oral administration of 2500 U (0.1 ml) daily. Calcium carbonate was dispensed as two chewable tablets that could be crushed and added to the child's food to provide 400 mg of elemental calcium daily. We assumed that daily calcium intakes above 500 mg would prevent rickets (400 mg supplement plus at least 100 mg in the diet). Pre-trial focus groups indicated that ground fish would be an acceptable dietary supplement. Local dried catfish (*Clarias gariepinus* or *Heterobranchus longifilis*) were baked and ground. A spoon was provided to administer 10 g of ground fish daily to be mixed with the child's food. Based on preliminary analysis, 10 g of ground fish was estimated to provide approximately 400 mg of elemental calcium. However, during the study, 20 monthly samples of ground fish were tested for calcium, and the mean (±SD) content of elemental calcium in 10 g of ground fish was 529±109 mg (courtesy of Michael Gruzak, USDA/ARS Children's Nutrition Research Center, Houston, TX). Samples of ground fish had no toxic concentrations of heavy metals.

Follow-up

Subjects returned every 4 weeks for their supplement. The volume of vitamin A, number of calcium tablets, and weight of ground fish remaining at each visit were recorded to assess adherence. Community workers attempted to contact those who failed appointments to encourage their return and identify reasons for missing appointments.

Height, weight, blood collection, and bone densitometry were repeated at 4, 8, 12, and 18 months after enrollment. Children who returned later than scheduled were tested when they returned with a minimum interval between measurements of 3 months.

After completing 18 months of follow-up, all children remaining in the cohort underwent repeat radiographs of the wrists and knees. All subjects, including those who had otherwise failed to continue in the trial, were encouraged to return for final evaluation and radiographs.

Statistical methods

The primary outcome was the development of rickets. Secondary outcome measures included serum calcium and alkaline phosphatase concentrations and areal bone density. We assumed that 9% of subjects in the vitamin A group [13] and less than 2% of subjects in each of the calcium supplementation groups would develop rickets. A sample of 100 children in each group would detect this difference with 95% confidence and 80% power. However, because it became clear that follow-up might not be as complete as we had initially assumed, we opened enrollment to all eligible subjects over a 5-month interval.

Statistical analyses were performed with SAS (version 9.1, SAS Institute, Cary, NC). Normally-distributed data are reported as means (±SD) and compared between groups with analysis of variance (ANOVA). Data that were not normally distributed are reported as medians with interquartile ranges and compared between groups with the Kruskal–Wallis test. Cumulative intake of supplement at the times of bone density and laboratory measurements was included in the analysis to reflect compliance. Repeated-measures analysis was used to compare mean values among treatment groups for follow-up time points after adjustment for differences in baseline characteristics. Multiple linear regression with PROC GENMOD and PROC MIXED was used to assess the effects of intervention group and age on bone mineral acquisition and biochemical variables. Significance of the interaction term of an intervention group with age in regression models was regarded as indicating the effect of the intervention over time on outcome measures. A two-tailed P value less than 0.05 was considered significant.

Besides calibration of stadiometers and weighing scales, we did not regularly assess the quality of height and weight measurements by study personnel. However, we examined the variation of anthropometric measurements between the three centers by statistical means. Based on standard growth charts, height and weight velocity are relatively constant between the ages of 12 and 36 months. With normal growth the standard scores (z-scores) of height for age and weight for age should remain relatively constant during this interval. Variation in standard score in an individual subject over the 18 months of measurements can be attributed to two factors. The first is variation in growth, which we assumed would be similar for all three groups. The second is variation in measurement technique. We attributed any significant

differences in the variance (*i.e.* the square of the standard deviation) of standard scores between the three groups to measurement variation.

Results

Study subjects

A total of 297, 187, and 229 children were screened for enrollment in the calcium tablet, ground fish, and control groups, respectively (Fig. 1). Active rickets was found radiographically in 7, 0, and 1 of the children in each of these groups, respectively (1.2% prevalence, 95% confidence interval 0.6 to 2.3%). Only two children with rickets had clinical features (enlarged wrists or curved legs) suggestive of the disease, while 10 children with clinical signs suggesting possible rickets had normal radiographs. Of 647 children enrolled in the treatment trial, 390 (60%) completed the final follow-up at 18 months (Fig. 1). The proportion who completed the trial did not differ significantly between the calcium tablet, ground fish, and control groups (57%, 63%, and 63%, respectively; $P = 0.28$).

Base-line characteristics of the three groups are shown in Table 1. Significant differences in age, family size, height-for-age, and all biochemical measurements were present between groups at enrollment. Daily dairy product calcium intake was negligible in the majority of subjects (median 0 mg, interquartile range 0–45 mg). The overall prevalence of vitamin D deficiency [serum 25(OH)D<12 ng/ml (30 nmol/l)] at baseline was 5.4% (95% CI 3.8%–7.7%). Serum 25(OH)D was over 20 ng/ml (50 nmol/l) in 49% (95% CI 45%–53%). Serum calcium and alkaline phosphatase concentrations were not significantly related to 25(OH)D values. Baseline 25(OH)D values were lower in the ground fish group (18 ± 6 ng/ml) than in the calcium tablet (22 ± 7 ng/ml) and placebo (23 ± 7 ng/ml) groups ($P<0.001$). Baseline 25(OH)D values were not related to enrollment age, gender, religion, height-for-age, breastfeeding status, or milk intake.

Results of treatment

Compared with those who completed the trial, subjects who dropped out came from larger families, discontinued breast feeding earlier, had fathers with occupations associated with lower incomes, and had greater baseline 25(OH)D values.

The mean ($\pm$SD) compliance as measured by the number of scheduled doses of vitamin A consumed divided by the number of person-days of follow-up was 57 ± 25, 65 ± 26, and 63 ± 31% in the calcium tablet, ground fish, and placebo groups, respectively ($P = 0.009$). Of those who returned for the 18-month follow-up visit, the total calcium consumed divided by the intended consumption was 50 ± 21% in the calcium tablet group and 57 ± 26% in the ground fish group ($P = 0.02$). The mean daily supplemental calcium consumed was 226 ± 99 mg in the calcium tablet group and 309 ± 137 mg in the ground fish group ($P<0.001$).

The median variance in height for age z-scores was significantly greater in the placebo group (0.49; IQR 0.17–1.47) than in the calcium tablet (0.29; IQR 0.10–0.53) or ground fish (0.24; IQR 0.11–0.45) groups ($P<0.001$). This likely indicates greater measurement error of height in the placebo group than in the groups that received calcium. The median variance in weight for age z-scores was similar in the placebo (0.35; IQR 0.22–0.65), calcium tablet (0.33; IQR 0.18–0.66), and ground fish (0.33; IQR 0.18–0.55) groups ($P = 0.43$).

Four new cases of rickets developed in 621 person-years of follow-up between 1 and 3 years of age. This corresponds to an approximate incidence rate of 6.4 cases per 1000 children per year. Two children in the placebo group, one child in the calcium tablet group, and one child in the ground fish group developed rickets (Table 2). The children in the calcium-supplemented groups who developed rickets had less than 50% adherence. The child who developed rickets in the calcium tablet group had a 25(OH)D <5 ng/ml at baseline and at 18 months, which is consistent with vitamin D deficiency at enrollment that was never resolved. Her initial radiographic score was 1.5, which was not enough to exclude her from enrollment, and 25(OH)D values were not available until completion of the study. She was the only subject with 25(OH)D <5 ng/ml at enrollment. The risk of developing rickets was not significantly increased with calcium adherence less than 50% ($P = 0.15$ by Fisher exact test).

Serum calcium did not differ between children in the three groups at the end of the study ($P = 0.70$). The relative risk of alkaline phosphatase values >350 U/L was unaffected by supplemental calcium (RR = 0.81, 95%CI 0.58–1.1). Overall, 5.4% (95%CI 3.8–7.7%) and 7.1% (95%CI 4.8–10%) had 25(OH)D values below 12 ng/mL (30 nmol/l) at baseline and 18 months, respectively. The change in 25(OH)D values was not affected by supplemental calcium ($P = 0.53$). Using 50% adherence as the cut-off

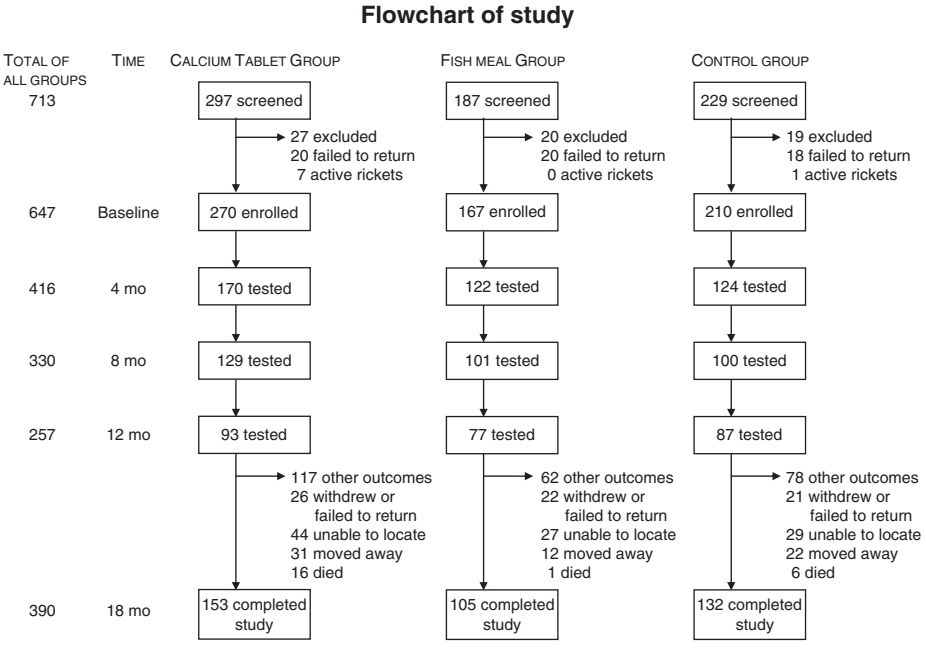

Fig. 1. Study flow.

Table 1
Comparison of base-line characteristics in children enrolled in three intervention groups.[a]

CHARACTERISTIC	GROUND FISH AND VITAMIN A (N = 167)	CALCIUM TABLETS AND VITAMIN A (N = 270)	VITAMIN A (N = 210)	P VALUE
Age (months)	14.0 ± 1.7	14.8 ± 2.1	14.6 ± 1.8	<0.001
Sex (M/F)	87/80	136/134	109/101	0.92
Religion (Christianity/Islam)	8/159	23/247	78/132	<0.001
Number of children in family	3 (2–5)	4 (2–6)	3 (1–5)	<0.001
First degree relative had clinical rickets (%)	14 (8.4%)	11 (4.1%)	11 (5.2%)	0.17
Breast feeding at enrollment (%)	159 (95%)	234 (87%)	188 (90%)	0.01
Age stopped breast feeding (months)[b]	19.6 ± 2.6 (N = 122)	18.8 ± 3.6 (N = 184)	18.3 ± 3.2 (N = 154)	0.001
Walking at enrollment (%)	63 (38%)	122 (45%)	104 (50%)	0.07
Age started walking (months)[b]	13.6 ± 2.8 (N = 142)	13.5 ± 3.4 (N = 212)	13.8 ± 3.5 (N = 179)	0.75
Milk product calcium intake (mg/d)[c]	0 (0–0)	6 (0–61)	13 (0–45)	<0.001
Socioeconomic status				
Father's occupational class score	4.9 ± 1.0	4.6 ± 1.3	4.4 ± 1.3	<0.001
Father's education (years)	6.8 ± 5.1	8.2 ± 5.1	8.6 ± 5.1	0.003
Mother's occupational class score	5.7 ± 0.7	5.5 ± 0.9	5.4 ± 1.0	0.01
Mother's education (years)	4.3 ± 4.2	5.0 ± 4.1	6.3 ± 5.0	<0.001
Anthropometric characteristics				
Height (cm)	72.0 ± 3.3	70.4 ± 4.5	68.6 ± 5.6	<0.001
Weight (kg)	8.2 ± 1.2	8.4 ± 1.4	8.5 ± 1.2	0.13
Height for age z-score	−1.9 ± 1.1	−2.7 ± 1.4	−3.3 ± 1.8	<0.001
Weight for age z-score	−1.9 ± 1.0	−1.9 ± 1.2	−1.8 ± 1.1	0.32
Weight for height z-score	−0.9 ± 1.1	−0.2 ± 1.2	0.7 ± 1.6	<0.001
Serum[d]				
Calcium (mg/dL)	10.19 ± 0.55	9.92 ± 0.77	10.06 ± 0.61	0.02
Corrected calcium (mg/dL)[e]	10.16 ± 0.41	10.07 ± 0.60	9.98 ± 0.42	0.002
Phosphorus (mg/dL)	5.71 ± 0.62	5.52 ± 0.79	5.68 ± 0.97	0.09
Alkaline phosphatase (U/L)	206 (171–241)	188 (155–236)	177 (148–221)	0.002
Albumin (g/dL)	4.02 ± 0.39	3.88 ± 0.47	4.07 ± 0.46	<0.001
25(OH)D (ng/mL) (range)	18.0 ± 5.7 (7–37)	22.2 ± 7.0 (2.5–37)	22.5 ± 7.3 (9–51)	<0.001
$1.25(OH)_2D$ (pg/mL)	120 ± 43	129 ± 49	141 ± 51	<0.001
Bone densitometry				
Distal radius and ulna				
Areal bone density (g/cm^2)	0.116 ± 0.020	0.119 ± 0.024	0.120 ± 0.021	0.36
Bone mineral content (g)	0.201 ± 0.047	0.202 ± 0.048	0.208 ± 0.047	0.27
Bone area (cm^2)	1.719 ± 0.186	1.690 ± 0.193	1.732 ± 0.213	0.07
Proximal 1/3 radius and ulna				
Areal bone density (g/cm^2)	0.184 ± 0.030	0.182 ± 0.033	0.185 ± 0.034	0.68
Bone mineral content (g)	0.293 ± 0.069	0.295 ± 0.077	0.299 ± 0.082	0.70
Bone area (cm^2)	1.581 ± 0.156	1.602 ± 0.196	1.600 ± 0.193	0.69

[a] Means are reported with ± SD. Non-normally distributed variables are reported as median values with interquartile range in parentheses.
[b] Determined from longitudinal data on the number of subjects indicated in parentheses.
[c] Excludes breast milk intake.
[d] To convert values for calcium to millimoles per liter, multiply by 0.25. To convert values for phosphorus to millimoles per liter, multiply by 0.32. To convert values for 25-hydroxyvitamin D to nanomoles per liter, multiply by 2.50. To convert values for 1,25-dihydroxyvitamin D to picomoles per liter, multiply by 2.40.
[e] Serum calcium was corrected for albumin concentration using the formula: Corrected calcium (mg/dL) = Total calcium (mg/dL) + 1.2 × [4 − albumin (g/dL)].

between adherent and non-adherent subjects in the calcium-supplemented groups, we found no significant differences in serum calcium, phosphorus, alkaline phosphatase, or 25(OH)D between adherent and non-adherent subjects at 18 months.

Compared with the group that received no calcium supplementation, areal bone density increased significantly more with time at both the distal and proximal 1/3 radius and ulna in the two groups that received calcium supplementation (Tables 3 and 4; Fig. 2). We explored several models that included different variables to examine their effect on the interaction of calcium supplementation with age. This interaction represented the effect of calcium supplementation on bone density over time compared with the group that did not receive calcium. By adjusting for the group assignment, we accounted for the baseline differences between the three groups in general. As the number of variables in the model increased, the significance of the interaction of calcium supplementation with age tended to increase, suggesting that baseline differences between the three groups may have diluted the observed effect of calcium supplementation. When the interaction terms are significant, as they are in the models we explored, the negative coefficients of the main effects (*i.e.* group assignment) do not affect the interpretation of the interaction terms. In this case, the coefficients of the main effects are not interpretable alone.

Parameter estimates for the interaction of calcium supplementation with age were significant for both calcium tablets and ground fish, indicating a greater increase in areal bone density with time in the groups that received calcium compared with the group that received no calcium supplementation. The relationship of the increase in areal bone density with age was similar in each of the models, indicating a consistent effect of calcium supplementation independent of other variables in the models. When biochemical parameters were entered into the model, they did not attenuate the effect of calcium supplementation on bone density with time.

In a model adjusted for age, height, alkaline phosphatase, calcium, and 25(OH)D, the relative increase in bone density at the distal radius and ulna compared with the placebo group over 18 months was 0.011 g/cm^2 (+8.5%) and 0.012 g/cm^2 (+9.2%) in the calcium tablet and ground fish groups, respectively. The relative increase in bone density at the proximal 1/3 radius and ulna compared with the placebo group was 0.011 g/cm^2 (+5.3%) and 0.018 g/cm^2 (+8.7%) in the calcium tablet and ground fish groups, respectively.

We examined the relationship of change in areal bone density with cumulative calcium intake in the two groups that received calcium supplements, adjusted for age, study group, and change in body weight. The dose–response relationship of calcium supplementation was not

Table 2
Characteristics of four children who had rickets at the final visit.

Intervention group	25(OH)D (ng/ml)	Calcium (mg/dL)	Alkaline phosphatase (U/L)	Forearm bone density*	Calcium adherence (%)	Vitamin A adherence (%)	X-ray score at 18 mo.
Calcium tablets	<5	9.3	1396	↓	47	48	8.0
Ground fish	14	9.4	625	↓	12	17	2.5
No calcium	13	9.1	336	↑	–	15	5.0
No calcium	9	9.5	1239	↓	–	94	3.0

* Relative to the median.

significant in the distal radius and ulna (P = 0.28), but it was significant in the proximal 1/3 radius and ulna (P = 0.04).

Since the areal bone density is a function of both projected bone area and bone mineral content, we further examined the effect of calcium supplementation on bone area and bone mineral content. In a multivariate model adjusted for age, sex, height, alkaline phosphatase, calcium, and 25(OH)D, the change in bone area at the distal and the proximal 1/3 radius and ulna with age was not significantly different in the groups that received calcium compared with the group that received no calcium supplementation. However, in a multivariate model adjusting for the same variables, the increase in bone mineral content with time was significantly greater in the groups that received calcium compared with the group that received no calcium (P<0.001 for calcium tablets and P = 0.001 for ground fish at the distal radius and ulna; P = 0.03 for calcium tablets and P<0.001 ground fish at the proximal 1/3 radius and ulna). Thus, calcium supplementation increases bone density by primarily increasing bone mineral content rather than through an effect on bone area.

In a model adjusted for age and sex, the gain in weight over time was not significantly different in the groups that received calcium tablets or ground fish (P = 0.71 and P = 0.38 for interaction, respectively) than in the group that received no calcium supplementation. Due to differences in variability in height measurements between the three groups, we did not assess the effect of calcium supplementation on height velocity.

Discussion

In this trial of calcium supplementation for prevention of rickets, we were unable to demonstrate a protective effect of calcium supplementation on the occurrence of rickets between the ages of 12 and 36 months. Bone density, however, did improve with calcium supplementation, as compared with placebo-treated children. The increase in forearm bone density was mediated by an increase in bone mineral content rather than through changes in projected bone area.

Several possibilities could explain the lack of an observed protective effect of supplemental calcium on the incidence of rickets. Because we observed only two cases of rickets in the control group (1.5%), our sample size had inadequate power to demonstrate a significant reduction in the incidence of rickets in the groups receiving calcium supplementation. Exclusion of eight cases (1.2%) of largely subclinical rickets at enrollment also contributed to a lower than expected number of cases of rickets. Adding excluded cases to the cases that developed during the study represents an incidence of rickets by age 3 years of approximately 1.7%. We cannot rule out an effect of vitamin A in preventing rickets, which could account for fewer than the expected number of cases of rickets in all groups. However, most evidence suggests that vitamin A potentiates the risk of rickets [14,15].

A major limitation of our study was the lack of randomization at an individual subject level. In order to simplify study logistics and avoid contamination, we provided the intervention to three similar, but geographically separate, urban communities in the same city. However, significant baseline differences were present between the three groups, and differences in unmeasured variables probably also existed. Although it is possible to control for measured factors in the analysis, it is not possible to adjust for residual confounding by variables that were not measured. An unmeasured factor in the control group could have been protective for rickets, accounting for a lack of observed effect for calcium supplementation.

We observed a modestly favorable effect of supplemental calcium on areal bone density. Each subject served as his or her own control in examining the effect of calcium supplementation, and a dose–response effect was observed with calcium supplementation in the proximal 1/3 radius and ulna. To our knowledge, this is the first study to report the effect of calcium supplementation on bone mineral density and acquisition in children less than 3 years of age. In children 3–5 years of age, calcium supplementation with 1000 mg/d for 12 months resulted in greater increases in bone mineral content and cortical thickness in the tibia that was dependent on physical activity [16]. In Gambian children, ages 8–12 years, calcium supplementation with 1000 mg/d increased mid-shaft radial bone density by 4.5% [17]. In 7 year-old Chinese children, calcium supplementation with 300 mg/d increased bone density in the radius by 3.1% [18]. In U.S. children 6–14 years of age, calcium supplementation with 1000 mg/d increased mid-shaft radial bone density by 5.1% [19]. We observed a relative increase in bone density in the proximal 1/3 radius and ulna of 5.3% and 8.7% in the calcium tablet and ground fish groups, respectively. The magnitude of change in bone density with calcium supplementation may be accentuated by the low calcium intakes in Nigerian children.

Table 3
Linear regression models of factors related to areal bone density ($g/cm^2 \times 1000$) of the distal radius and ulna.*

Characteristic	Model A estimate (P value)	Model B estimate (P value)	Model C estimate (P value)	Model D estimate (P value)
Group†				
Calcium tablet	1.0 (0.57)	−9.7 (0.01)	−9.1 (0.03)	−12.4 (0.008)
Ground fish	−2.4 (0.20)	−11.6 (0.005)	−13.9 (0.001)	−18.1 (<0.001)
Age (months)	−0.51 (<0.001)	−0.84 (<0.001)	−0.78 (<0.001)	−0.94 (<0.001)
Height (cm)	1.4 (<0.001)	1.4 (<0.001)	1.4 (<0.001)	1.7 (<0.001)
Female sex		−3.0 (0.05)		
Alkaline phosphatase (U/L)			−0.006 (0.29)	−0.018 (0.03)
Calcium (mg/dL)			4.7 (<0.001)	4.2 (0.02)
25(OH)D (ng/mL)				0.18 (0.08)
Interactions				
Calcium tablet × age		0.50 (0.005)	0.50 (0.008)	0.62 (0.002)
Ground fish × age		0.43 (0.02)	0.55 (0.005)	0.67 (0.001)

* Estimates were based on generalized estimating equations with adjustment for repeated measures.
† Referent group is the one that received no calcium supplementation.

Table 4
Linear regression models of factors related to areal bone density (g/cm^2 × 1000) of the proximal 1/3 radius and ulna.*

Characteristic	Model A estimate (P value)	Model B estimate (P value)	Model C estimate (P value)	Model D estimate (P value)
Group†				
Calcium tablet	0.75 (0.76)	−9.8 (0.08)	−10.4 (0.07)	−13.7 (0.02)
Ground fish	0.92 (0.73)	−13.7 (0.02)	−18.5 (0.002)	−22.0 (0.001)
Age (months)	−0.26 (0.20)	−0.67 (0.01)	−0.59 (0.04)	−0.69 (0.04)
Height (cm)	1.9 (<0.001)	1.9 (<0.001)	2.0 (<0.001)	2.4 (<0.001)
Female sex		−2.4 (0.25)		
Alkaline phosphatase (U/L)			−0.025 (0.01)	−0.041 (<0.001)
Calcium (mg/dL)			7.4 (<0.001)	8.2 (<0.001)
25(OH)D (ng/mL)				0.26 (0.15)
Interactions				
Calcium tablet × age		0.49 (0.04)	0.56 (0.02)	0.61 (0.01)
Ground fish × age		0.69 (0.007)	0.94 (<0.001)	1.0 (<0.001)

* Estimates were based on generalized estimating equations with adjustment for repeated measures.
† Referent group is the one that received no calcium supplementation.

Compliance and calcium intake were significantly greater in the ground fish group than in the calcium tablet group, perhaps indicating a greater acceptance of familiar foods compared with supplemental medication.

In conclusion, we could not demonstrate that calcium supplementation prevented nutritional rickets in young Nigerian children, but it improved bone density. An affordable and available food-based source of calcium was readily accepted, and future studies may find it advantageous to focus on food-based sources of improved calcium nutrition. As the study was underpowered to assess the effect calcium supplements on the incidence of rickets, further studies are needed to confirm this finding.

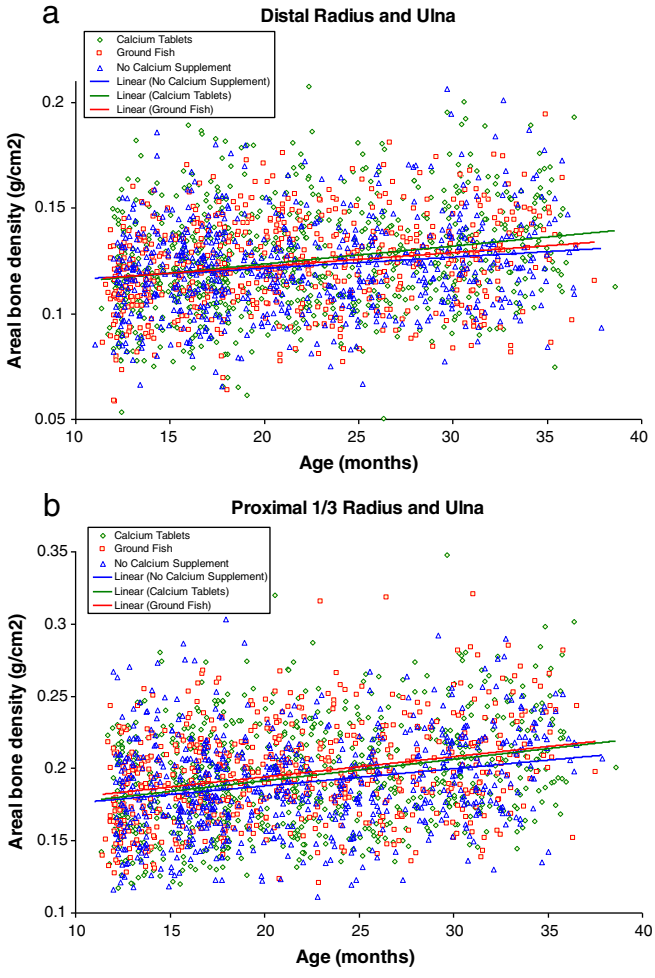

Fig. 2. Relationship of areal bone density of the distal (a) and proximal 1/3 (b) radius and ulna with age in the three groups. Data from all children returning for follow-up visits are included.

Conflict of interest

The authors declare no conflict of interest.

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