Adaptation of calcium absorption during treatment of nutritional rickets in Nigerian children

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Nutritional rickets in Nigerian children has been effectively treated with Ca supplementation. High values of Ca absorption efficiency have been observed in untreated children, but whether Ca absorption efficiency changes during treatment with Ca is unknown. Our objective in conducting this study was to identify the effect of Ca therapy on Ca absorptive efficiency in children with primary Ca-deficient nutritional rickets. Twelve children with radiographically active rickets, 2 to 14 years of age (median 39 months) participated in the study. We assessed dietary Ca intake via dietary recalls, and measured biochemical markers of Ca and vitamin D homeostasis. Fractional Ca absorption was measured using a dual tracer stable isotope method, before and after 2 weeks of treatment with 15·0 mmol elemental Ca daily. Ten children had adequate urine collection for inclusion in the analysis. Usual dietary Ca intake was 4·2 (SD 1·0) mmol/d. The median Ca absorption prior to treatment was 72 % (range 52–97 %) and decreased significantly to 57 % (31–84 %) (*P*=0·004) after 2 weeks of supplementation. We conclude that Nigerian children with rickets adapt to Ca supplementation with a small decrease in Ca absorptive capacity, but retain very high absorptive levels during supplementation. Overall Ca absorption efficiency was comparable with that identified in other populations with low Ca intakes. These data demonstrate that although absorptive capacity is regulated by supplementation, recovery from rickets likely occurs through efficient use of both dietary and supplemental Ca.

Stable isotopes: Africa: Dietary Ca deficiency: Intestinal Ca absorption

Nutritional rickets continues to be a major paediatric concern in many developing countries including Nigeria, Bangladesh and Mongolia. While the aetiology of nutritional rickets in young children has usually been ascribed to vitamin D deficiency associated with prolonged breast feeding and lack of sunlight exposure or vitamin D supplementation, prior studies in Nigerian children suggest that inadequate Ca intake is the principal cause of nutritional rickets in this population[1–3]. Ca supplementation, with or without vitamin D, produces healing of rickets in Nigerian children[4,5]. Limited Ca intake by children in the developing world often results from a lack of dairy products in the customary diet. Most people rely on grains, legumes and vegetables to meet the needs of the growing skeleton for Ca. In addition, these customary diets often contain potential inhibitors of Ca absorption, such as phytates and oxalates. Consequently, the average daily Ca intake is commonly below 7·5 mmol for children in developing countries.

Ca absorptive efficiency is regulated by Ca intake. This occurs by multiple mechanisms. Low Ca intakes lead both to increased vitamin D conversion to the active 1,25-dihydroxyvitamin D form and to increased passive absorption of available Ca. Nonetheless, in the presence of high levels of dietary inhibitors and very low intakes, these mechanisms are inadequate to provide adequate Ca for skeletal growth. Although absorption efficiency is inversely related to the Ca load during a meal, little is known about the regulation of absorption efficiency over a period of supplementation in populations with usual low intakes. Concern may exist that decreased absorption would limit the efficacy of such interventions.

Clinical studies have demonstrated the efficacy of Ca supplementation in resolving rickets[4,5]. Thus, the use of supplements must lead to the absorption of enough bioavailable Ca to be beneficial. However, in designing intervention strategies, the effect of supplementation on the absorption of Ca

from the diet must be considered and there are no data on this relationship.

The aim of the present study was to identify the effects of Ca therapy on Ca absorption efficiency in Nigerian children with nutritional rickets. We hypothesized that the high values of Ca absorption efficiency prior to the treatment of rachitic children would be maintained after supplemental Ca therapy.

Experimental methods

We recruited seventy-three children with bowleg or knock-knee deformities characteristic of rickets by means of posters and word of mouth in the city of Jos, Nigeria. Based on our previous study in Nigerian children, we anticipated the mean fractional Ca absorption would be approximately 60 (SD 12) % at both the baseline and follow-up assessments[6]. We assumed the smallest clinically important difference in fractional Ca absorption to be 10 % between the baseline and 2-week assessments. A sample size of ten subjects would provide 80 % power to detect such a difference with 95 % confidence. Fifteen children were eligible and twelve children were enrolled to allow for possible data loss.

All subjects were interviewed and examined by one of the investigators and radiographs of the wrists and knees were obtained at the Jos University Teaching Hospital. Active rickets was defined as a radiographic score of at least 2 on a 10-point scale, where a score of 10 indicates the most severe rickets[7]. Children were offered enrolment if they had radiographically active rickets and the parent reported adequate bladder control, which was deemed necessary for a 24 h urine collection. Subjects were excluded if they had a chronic illness or were taking medications that might affect bone metabolism, including vitamin or mineral supplements. Informed written consent for the current study was obtained from a parent and the study was approved by the Investigational Review Board for Human Studies of Baylor College of Medicine and the Ethical Committee of Jos University Teaching Hospital. Subjects and their families were not given monetary compensation for their participation.

Baseline Ca intake was assessed from 24-h dietary recalls performed on two different days. We used a database constructed from food composition tables designed for African foodstuffs[8,9]. We measured each child's weight, height, triceps skinfold thickness and mid-upper arm circumference. Bone density of the forearm was measured with a Norland pDEXA (Model 476A110; Fort Atkinson, WI, USA) portable bone densitometer. Measurement sites included the area of minimal bone density of the ultradistal radius and ulna (primarily trabecular bone) and the distal one-third of the radius and ulna (primarily cortical bone). The instrument was set at standard precision and calibrated daily. Long-term precision of bone mineral content and areal density of a standard bone phantom were ± 0·017 g and ± 0·011 g/cm^2, respectively.

After an overnight fast, we admitted children to the Jos University Teaching Hospital for measurement of fractional Ca absorption using an established dual stable isotope technique[10] (Fig. 1). Topical lidocaine/prilocaine (2·5 %/2·5 %) anaesthetic cream was applied over a peripheral arm vein at least 60 min prior to insertion of an intravenous butterfly needle. During the 60 min after application of the topical anaesthetic, each child voided to empty their bladder, then ate a typical Nigerian breakfast of maize porridge (pap), to which 0·5 mg/kg ^{44}Ca and 2·5 mmol elemental Ca was added as calcium carbonate.

The Ca content of the entire breakfast with the added Ca was estimated to be approximately 4·5 mmol. This intake was used for both the pre- and post-supplementation study. Thus, the Ca intake during the pre-supplementation study day itself was higher than the usual daily intake, but well below that during the Ca supplementation period. This approach provided a consistent evaluation of the Ca absorptive capacity of the subjects before and after the supplementation period.

Blood was collected for measurement of serum Ca, P, albumin, 25-hydroxyvitamin D, alkaline phosphatase activity and parathyroid hormone (PTH). Subsequently, 0·12 mg/kg ^{42}Ca in 2 ml normal saline was given intravenously between 30 min and 2 h after ingestion of the oral isotope. All isotope samples were pre- and post-weighed to ensure accurate determination of isotope quantities. Urine was collected for 24 h following isotope administration. Total urine volume was recorded and 300 ml aliquots were collected from each child's pooled urine, frozen at −20°C and transported along with the frozen sera to Baylor College of Medicine for analysis.

Following completion of the baseline 24 h urine collection, all enrolled children were given chewable calcium carbonate tablets to provide 15·0 mmol elemental Ca daily to be taken orally in two divided doses at meal times for the next 14 d. After 14 d of Ca supplementation, enrolled children were readmitted for a second Ca absorption measurement. Compliance with Ca supplementation was assessed with pill counts. The procedures for isotope administration, serum collection and 24 h urine collection were identical to those of the baseline study. Children were not given the 15 mmol Ca supplementation during either admission, but only the 2·5 mmol with breakfast (yielding a total Ca intake of approximately 4·5 mmol when including the Ca present in the meal).

Ca therapy was continued for 6 months to allow time for complete healing of rickets to occur. Following 3 months of treatment, radiographs of the wrists and knees were repeated and bone density was measured.

All data were entered and analysed in Epi Info 2002 (CDC, Atlanta, GA, USA). Anthropometric indices were calculated with the nutritional anthropometry program of Epi Info using the CDC 2000 growth reference curves. Mean values are reported for normally distributed variables and median values for non-normally distributed variables. Correlation coefficients were calculated to quantify relationships between continuous variables. To compare mean values at different time points, the paired *t* test in Excel 2003 (Microsoft, Redmond, WA, USA) was used for all comparisons other than the comparison of radiographic rachitic severity at baseline and 3 months, in which case the Wilcoxon rank test was used. All *P* values quoted are for two-tailed tests.

Results

A total of seventy-three children with clinical characteristics of rickets (primarily leg deformities) underwent radiography of the wrists and knees. Of the seventy-three children with clinical rickets, fifteen had radiographically active rickets and twelve of these were enrolled. The three children who were not enrolled either did not have good bladder control or did
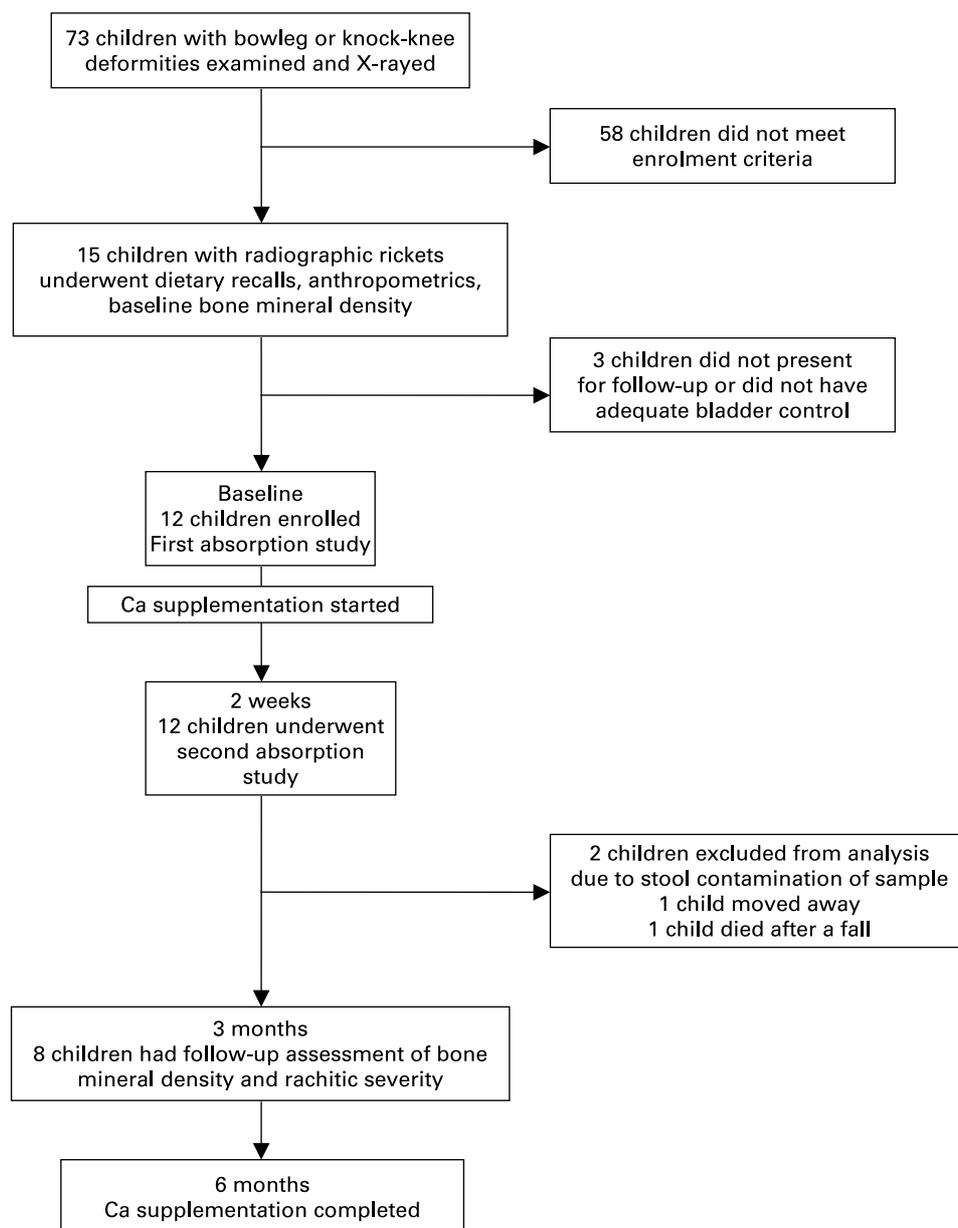
Fig. 1. Study profile. For details of subjects and procedures, see Experimental methods.

not return for follow-up appointments. Two enrolled children were excluded from the final analysis because their urine collections were poor (excess spillage or stool contamination), and one of them probably had incomplete intake of the oral isotope (she did not want to complete the maize pap containing the isotope). Characteristics of the remaining ten study subjects are shown in Table 1. One child died from a fall 2 months after enrolment and another moved away, accounting for the loss of two children at the 3-month follow-up visit.

The ages of most subjects were between 2 and 5 years (median age of 39 months, range 25 to 175 months), except for one 6 and one 14 year-old. None of the children was currently breast feeding. The baseline dietary Ca intake of the children was low with a mean daily Ca intake of 4·2 (SD 1·0) mmol. There was a trend for baseline values of 25-hydroxy-vitamin D to be inversely related to the radiographic rickets

score (r −0·63; P=0·06), although this relationship did not show statistical significance. There was also no relationship between values of 25-hydroxyvitamin D and forearm bone mineral density.

Baseline fractional Ca absorption efficiency was high, with a median value of 72 % (range 52–97 %) and trended towards a significant inverse relationship to age (r −0·60; P=0·07). The relationship of absorption with age was significant when the logarithm of age was used (r −0·69; P=0·03). Baseline Ca absorption was significantly positively related to the weight for age z-score (r 0·64, P=0·05) but not to the weight for height or height for age z-scores. Baseline Ca absorption was inversely related to bone mineral density (r −0·71, P=0·02 for the ultradistal wrist and r −0·64, P=0·05 for the distal one-third wrist). There was no significant relationship of baseline fractional Ca absorption with

Table 1. Characteristics of ten Nigerian children with nutritional rickets†

(Median values and range for variables with skewed distribution. Mean values and standard deviations for variables with normal distribution)

Characteristic	Baseline (n 10)			3-month follow up (after Ca supplementation) (n 8)			P value*
	Mean	SD	Range	Mean	SD	Range	
Age (months)	38·7		24·7–175				
Sex							
Male	5						
Female	5						
Weight for age z-score	−1·5		−4·2 to −0·8	−1·7		−3·7 to −1·1	0·89
Weight (kg)	14·2	6·2		15·7	6·9		
Height for age z-score	−2·7		−4·3 to −0·9	−2·7		−3·5 to −1·0	0·37
Weight for height z-score	−0·5	1·0		−0·2	1·0		0·74
BMI (kg/m²)	16·1	1·3		16·0	1·2		0·32
MAC for age z-score	−1·2	1·0					
Dietary Ca intake (mmol/d)	4·2	1·0					
Dietary Ca intake per kg body weight (mmol/kg per d)	0·3	0·1					

MAC, mean arm circumference.
*P values for the 3-month follow-up are only for the baseline v. 3-month comparison of eight children.
† For details of subjects and procedures, see Experimental methods.

dietary Ca intake (r 0·04, $P = 0.91$), sex (females 76 % and males 70 %, $P = 0.64$), or radiographic score (r −0·03, $P = 0.94$). Furthermore, baseline fractional Ca absorption was also not related to serum 25-hydroxyvitamin D (r 0·22; $P = 0.54$) or PTH values (r −0·37; $P = 0.29$) and PTH values were unrelated to 25-hydroxyvitamin D values.

After treatment with 15 mmol elemental Ca daily over a 2-week period, the median fractional Ca absorption efficiency decreased from 72 % (range 52–97 %) at baseline to 57 % (range 31–84 %) (Table 2). The mean decline in fractional absorption was 20 (SD 18) % (range −0·4 to −58 %). The change in Ca absorption over the 2-week interval was not significantly related to age, sex, anthropometric indices, baseline Ca absorption, serum Ca, alkaline phosphatase activity, 25-hydroxyvitamin D, PTH, bone density at the wrist or severity of radiographic score. The baseline low-normal serum Ca, low serum P and elevated alkaline phosphatase activity values were consistent with nutritional rickets. These values did not change significantly during the initial 2-week treatment interval (Table 2).

Treatment compliance ranged from 82 to 96 % of the supplied Ca tablets over the 3 months of follow up. In the subjects who had bone densitometry at the 3-month follow up, there was no relationship between the fractional Ca absorption at 2 weeks and the increase in bone mineral content or density (Table 2). However, there was a trend toward a greater increase in distal one-third radius and ulna bone mineral content among those with less severe baseline radiographic scores (or a lower score on the 10 point rickets radiographic scale) (r −0·74; $P = 0.07$), although these results were not statistically significant. This increase in bone density was unrelated to baseline alkaline phosphatase activity or to improvement in radiographic score with treatment.

Discussion

Children with rickets require much longer than 2 weeks for their bones to become mineralized and the metabolic demand for Ca should remain high until the bones achieve nearly complete mineralization. We found that within 2 weeks fractional Ca absorption decreased during Ca supplementation in Nigerian children with nutritional rickets. These findings are consistent with the known effects of increasing Ca intake on fractional Ca absorption in healthy adults and children[11,12]. Ca absorption in healthy adults has both a component that is saturable (vitamin D dependent) and a component proportional to intake[13]. The active component of Ca absorption is saturable and down regulated when Ca intake increases[14]. A possible explanation for the present findings of a reduction in fractional Ca absorption associated with Ca supplementation is that the active component of Ca absorption is down regulated in response to the reduction in 1,25-dihydroxyvitamin D concentration following improved Ca balance. Nigerian children with rickets therefore quickly adapt to an increased Ca intake. Active Ca transport in the duodenum is known to be down regulated when Ca intake increases[14]. By increasing Ca intake, the total amount of Ca absorbed increases, even though the fractional absorption declines.

In adolescent girls, an increased Ca intake (21·2 (SD 2·0) mmol/d compared with 47·4 (SD 1·2) mmol/d) reduced bone resorption by 32 %, resulting in enhanced retention of Ca in bone[15]. However, the fractional absorption of Ca in these girls did not change over a 1-month interval. This lack of change in fractional Ca absorption may be because the lower Ca dose of 21·2 (SD 2·0) mmol/d is already relatively high (unlike in the current study) and fractional Ca intake was already relatively low at 48 %, leaving little room for a further decrease in fractional Ca absorption. These results differ from those of a study in young children (3–5 years old), which are consistent with the present findings, and demonstrated a 12·5 % decline in fractional absorption when dietary Ca intake was increased more than two-fold[16]. This decline in fractional Ca absorption might be explained by the effect that doubling the Ca content of the test meal had on the intestinal Ca absorption process, which probably

Table 2. Impact of Ca supplementation on Ca absorption efficiency and other markers of rickets in ten children†

(Median values and range for variables with skewed distribution. Mean values and standard deviations for variables with normal distribution)

Characteristic	Baseline (n 10)			After 2 weeks of Ca (n 10)			3-month follow up (n 8)			P value*
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
Median Ca absorptive efficiency (%)	72		52–97	57		31–84				0·004
Radiographic rachitic severity‡	4·4	2·4					3·1	2·3		0·002
Bone densitometry										
Ultradistal radius and ulna										
Areal bone density (g/cm²)	0·129		0·106–0·216				0·162		0·119–0·240	0·04
Bone mineral content (g)	0·272		0·192–0·311				0·334		0·268–0·596	0·007
Bone area (cm²)	2·10		1·81–2·72				2·11		1·99–2·89	0·007
Distal 1/3 radius and ulna										
Areal bone density (g/cm²)	0·210		0·154–0·370				0·238		0·162–0·394	0·24
Bone mineral content (g)	0·382		0·261–0·822				0·409		0·293–0·889	0·40
Bone area (cm²)	1·89		1·69–2·43				1·89		1·72–2·50	0·99
Serum										
Ca (mmol/l)	2·14	0·14		2·11	0·19					0·36
P (mmol/l)	0·92	0·20		0·93	0·19					0·63
Alkaline phosphatase (U/l)	735	229		798	311					0·34
25-Hydroxyvitamin D (μmol/l)	39·5	12·5								
PTH (pg/ml)	292	156								

PTH, parathyroid hormone.

*P values for the 3-month follow-up are only for the baseline v. 3-month comparison of eight children.

† For details of subjects and procedures, see Experimental methods.

‡ Based on a validated 10-point score with 10 representing the most severe rickets (comparison based on Wilcoxon rank test).

switched from being a purely active process to one that was dependent on both active and passive processes[16]. Also consistent with the present findings are results from a study of African American and Caucasian pubertal girls, which found that fractional Ca absorption decreased from 63·8 % on a low Ca intake of 9·7 mmol/d to 44·9 % on a high Ca intake of 31·5 mmol/d[17].

The high values of fractional Ca absorption in our subjects indicate that 1,25-dihydroxyvitamin D was sufficient to stimulate Ca absorption despite less than optimal 25-hydroxyvitamin D status. The high values of fractional Ca absorption that we observed may result not only from rachitic disease associated with high 1,25-dihydroxyvitamin D concentrations in our subjects but also from ethnic factors. Despite lower values of 25-hydroxyvitamin D, fractional Ca absorption has been found to be greater in black than in white girls between the ages of 5 and 16 years[18]. In studies of children in both the United States and Nigeria, we have observed no relationship between 25-hydroxyvitamin D concentrations and fractional Ca absorption[6,19]. The fractional absorption values in this study are similar to those we have found previously in Nigerian children, suggesting a maximal absorption fraction of about 70–80 %, regardless of 25-hydroxyvitamin D values. The lack of a relationship between baseline fractional Ca and 25-hydroxyvitamin D level in the present study is also consistent with the previous study[6].

In our previous study, baseline fractional Ca absorption in children with rickets and in matched control children was high (61 % and 63 %, respectively)[6]. After 12 months following treatment with Ca and documented healing of rickets, mean values of fractional Ca absorption (81 %) in rachitic children were significantly greater than those prior to treatment. However, unlike the current study, the children with healed rickets were not taking supplemental Ca at the time

of the 12-month study. Interestingly, the severity of rickets did not correlate with higher baseline fractional absorption. For ethical reasons, our current study was not performed with a control group of children with rickets who did not receive treatment for the duration of the study period; therefore, the observed decrease in fractional Ca absorption may be secondary to confounding factors that change the measurement of Ca absorption over time.

The body adapts to an increased Ca intake by down regulating Ca absorptive efficiency to less than the presumably maximal values observed prior to treatment. Nigerian children with rickets are likely to adapt quickly to an increased Ca intake. Active Ca transport in the duodenum is known to be down regulated when Ca intake increases[18]. Chinese children with daily Ca intakes of 12·5 mmol or less had higher fractional Ca absorption than those with daily intakes over 12·5 mmol (63 % and 55 %, respectively)[20]. In another study by the same author, baseline fractional Ca absorption declined from 61 % to 56 % after 6 months of Ca supplementation in Chinese children, but the difference was not significant[21]. It is important to note that although the fractional Ca absorption declined over the 2-week treatment interval in our subjects, the estimated total amount of Ca absorbed increased more than three-fold due to the increased Ca intake associated with the addition of Ca supplements.

We did not find any relationship between the fractional Ca absorption at 2 weeks and the increase in bone mineral content or density for the eight subjects who were available for the 3-month follow up, although there was a statistically insignificant trend towards increased bone mineral content in those children whose rickets was less severe by our radiographic parameters. We did find that, overall, these eight children had a statistically significant improvement in their radiographic rickets severity score after 3 months of daily Ca

supplementation (4.4 ± 2.4 to 3.1 ± 2.3, $P < 0.001$) as well as improvement in the bone mineral density and bone mineral content of the ultradistal radius and ulna (Table 2). These findings might imply that Ca supplementation ultimately improves the course of rickets; however, we are not able to say that this improvement in rickets is related to the level of fractional Ca absorption. With only eight subjects left at the 3-month follow up there is the possibility that we did not have the power to detect a relationship if one truly did exist.

We cannot determine the exact absorptive efficiency from the Ca supplement itself. Since the supplement is not taken with dietary inhibitors such as might be found in usual Nigerian meals and the supplement is given over the span of the day, it is likely that absorption efficiency is high. Even if the absorption efficiency of the 15·0 mmol supplement is 20 % less than the usual Ca absorption, the total amount of Ca absorbed would more than double from the pre-supplement total Ca absorbed.

Also, the extent to which our measured absorption efficiency from the test meal represents a child's fractional Ca absorption is based on the extent to which the Ca proportion in our test meal is representative of the average Ca proportion in that child's daily diet. The test meal we provided was higher than the children's average baseline Ca intake, but still very low in Ca and likely reasonably represented usual Ca intake. Because most of the Ca in the diet of children in Nigeria is in the form of plant foods and grains, there is no way to accurately trace this intake and thus a true absolute measure of absorption is not possible from the food sources without intrinsic labelling of the food sources.

In conclusion, we found that Nigerian children with nutritional rickets decrease their Ca absorptive efficiency slightly in response to Ca supplementation. However, the overall maintenance of a high efficiency of Ca absorption in the face of supplementation indicates that such supplementation strategies do not impair the native absorptive capacity and that children with rickets maintain a normal pattern of Ca homeostasis.

Acknowledgements

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