

ORIGINAL ARTICLE

Calcium intake of rural Gambian infants: a quantitative study of the relative contributions of breast milk and complementary foods at 3 and 12 months of age

LMA Jarjou¹, GR Goldberg^{1,2}, WA Coward^{2,*} and A Prentice^{1,2}

BACKGROUND/OBJECTIVES: There is a paucity of information from developing countries on total calcium intake during infancy, and potential consequences for growth and bone development.

DESIGN: Observational longitudinal study of rural Gambian infants (13 males and 17 females) at 3 and 12 months of age.

SUBJECTS/METHODS: Breast-milk intake and calcium concentration, weighed dietary intake, anthropometry, midshaft radius bone mineral content (BMC) and bone width (BW).

RESULTS: At 3 and 12 months (mean \pm s.d.) calcium intake from breast milk was 179 ± 53 and 117 ± 38 , and from other foods 12 ± 38 and 73 ± 105 mg/day. There was no difference in total calcium intake; 94% and 62% of calcium came from breast milk. At 3 and 12 months, weight s.d.-scores were -0.441 ± 1.07 and -1.967 ± 1.06 ; length s.d.-scores were -0.511 ± 1.04 and -1.469 ± 1.13 . Breast-milk calcium intake positively predicted weight ($P = 0.0002$, $P \leq 0.0001$) and length ($P = 0.056$, $P = 0.001$). These relationships were not independent of breast-milk intake, which positively predicted weight ($P \leq 0.002$) and length ($P = 0.06$, $P = 0.004$). At 3, but not 12 months, weight and length correlated with total calcium intake. There were no relationships between total calcium intake and breast-milk intake with BW or BMC.

CONCLUSION: The combination of low calcium intake from breast milk and complementary foods resulted in a low total calcium intake close to the estimated biological requirement for bone mineral accretion. Relationships between calcium intake and growth were largely accounted for by breast-milk intake, suggesting that low calcium intake *per se* was not the limiting factor in the poor growth. These findings have potential implications for deriving calcium requirements in developing countries.

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INTRODUCTION

Worldwide ~ 200 million children < 5 years of age are affected by poor growth and stunting (height-for-age > 2 s.d. below reference population mean). There are many causes including poor nutrition and micronutrient deficiencies during infancy. Adverse outcomes include higher morbidity and mortality, impaired immune function and reduced cognitive function.¹

Six months exclusive breastfeeding is recommended worldwide.² Prolonged exclusive breastfeeding can potentially cause energy and nutrient deficiencies when introduction of complementary foods is inappropriately delayed.^{3–5} However, many complementary foods in developing countries have low nutrient density, often containing only small amounts of calcium and other minerals needed for bone growth and development.⁶ Furthermore, the mineral content of breast milk declines as lactation proceeds.^{7,8}

Thus, prolonged breastfeeding combined with complementary foods of poor nutrient quality may lead to inappropriately low calcium intakes, and be an important contributory factor to poor growth.⁶ However, there are few quantitative data for basing

judgements about the adequacy of calcium intake where stunting is common, and no published studies of calcium intake from breast milk and foods in the same individual. This study was performed in rural Gambia where breastfeeding is universally practiced for 18–24 months, and complementary foods of limited variety and quality are typically introduced from 3–4 months. Calcium densities in breast milk,⁹ complementary and most adult foods^{6,10} are low. Poor growth and bone development are common features of infancy and early childhood.^{9,11} The aim of this study was to fully quantify calcium intakes of individual infants and explore relationships with measures of growth and bone mineral accretion.

SUBJECTS AND METHODS

Subjects

Participants were 30 mothers and infants living in two villages, Keneba and Manduar, between 1990–1992, studied 3 and 12 months after delivery. They were a subset of 60 pairs participating in a randomized, placebo-controlled study of maternal calcium supplementation during lactation.

¹MRC Keneba, Fajara, The Gambia and ²MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, UK. Correspondence: Dr LMA Jarjou, MRC Keneba, PO Box 273, Fajara, The Gambia.

E-mail: ljarjou@mrc.gm

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A total of 15 mother–infant pairs from each arm were randomly selected. The supplement was subsequently shown to have had no effect on breast-milk calcium concentration or intake.¹⁰ The study was explained to the women in their local language and written consent obtained. Approval was given by the joint MRC/Gambian Government Ethics Committee. Samples (see below) were stored at -20°C immediately after collection and later transported frozen to Cambridge (UK), for analysis.

Breast-milk intake

Breast-milk intake was measured using a stable isotope tracer technique, ('dose-to-the-mother-method'), currently the gold standard method for such determinations.^{12–14} On day 0, an early morning breast-milk sample was collected from the mother, and saliva from the infant to provide data on baseline isotopic composition. To minimize breast-milk contaminating saliva, samples were collected at least 1 h after the infant's last feed. Breast milk (1.0 ml) was expressed by the mother into low calcium tubes (Z5 tubes; Bibby Sterilin, Stone, UK). Saliva (0.5 ml) was collected by gently rubbing the infant's gum with cotton wool, which was later put into a syringe and the saliva squeezed into a 0.5 ml eppendorf tube (Sarstedt Ltd, Leicester, UK). The mother was then given an accurately weighed dose of 100 mg D_2O /kg body weight. Subsequent breast-milk and saliva samples were collected 1, 2, 3, 13 and 14 days after dose. Mothers and infants were weighed on days 0 and 14.

Thawed breast milk was left at 37°C for 30 min, microcentrifuged for 5 min and then defatted by gentle aspiration. The water in breast milk and saliva was reduced to hydrogen gas over hot zinc before determination of deuterium enrichment using mass spectroscopy (SIRA 10, VG Isotech, Middlewich, UK). Breast-milk intake was estimated from a two-compartmental model, describing the observed mono-exponential decay curve of deuterium in milk and bi-exponential curve of deuterium enrichment in saliva.¹³ This model treats maternal and infant body water as single pools, with unidirectional flow from mother to baby, representing the volume of breast-milk intake. Mass spectrometry is the reference method for stable isotope determination, and there have been no methodological developments in the last few decades, which have improved the accuracy or precision of deuterium analysis.

Breast-milk calcium concentration and intake

Breast milk for calcium analysis (1–2 ml) was manually expressed from each breast by the mother and collected as described above. Time of day and stage of feed was unspecified because previous work in this population demonstrated no significant influence on calcium concentration.⁹

Samples and standards were analyzed in duplicate using an established semi-micro method that had been validated against atomic absorption spectrometry.¹⁵ Average results from samples collected from both breasts were used. Breast-milk calcium intake (mg/day) was calculated by multiplying breast-milk intake (l/day) by calcium concentration (mg/l).

Calcium intake from complementary foods

A prospective 5-day record was obtained by direct weighing to determine food intake from any sources other than breast milk.⁶ Field staff visited the infant at home several times a day to record the infants' intake. Mothers were asked to keep to their usual habits with respect to preparing and offering food to their infant. Foods, including snacks (small tastes of foods), milks and other drinks, were weighed by field staff before the mother fed her infant, and any leftovers also weighed. All recipes were recorded. Calcium intake was calculated from the database of Gambian foods and in-house coding and analysis programmes held at MRC Human Nutrition Research.¹⁶ Intake from village drinking water was not quantified because it contains little calcium ($<10\text{ mg/l}$).⁶ Details of breast-feeding practice, including whether infants received drinking water was collected by questionnaire.

Anthropometry

Infant weight and length was measured to the nearest 0.01 kg and mm using Seca scales and Kiddimetre length board, respectively (Raven Equipment Ltd, Dunmow, UK).

Bone mineral status

Bone mineral content (BMC; g/cm), bone width (BW; cm) and bone mineral density (BMD; g/cm^2) at the midshaft radius were measured by single-photon absorptiometry (Norland 2780, Fort Atkinson, WI, USA).

The instrument was calibrated prior to each measurement. A reference phantom of bone mineral containing regions representing 0.54 and 1.52 g/cm was scanned regularly. Between-day precision was 1.3% and 1.0%, respectively.

Statistics

DataDesk 6.0 software (Data Description Inc., Ithaca, NY, USA) was used for Student's *t*-test, correlation, multiple regression, analysis of variance and covariance and linear models. The significant threshold was set at $P=0.05$. Summary statistics in the text and tables are presented as mean \pm s.d. for normally distributed data, and median and interquartile range (IQR) for data with positively skewed distributions.

S.d.-scores were calculated for weight and length relative to British reference infants¹⁷ and to WHO growth standards.¹⁸

Multiple regression models were constructed to investigate relationships between calcium intake and weight, length and bone variables. A sex term was included when appropriate. Non-significant variables were excluded by backwards elimination, removing the least significant first. BMC was adjusted for BW and body size (weight, length) to give size-adjusted BMC (SA-BMC) using multiple regression.

Paired Student's *t*-test was used to test significance of individual changes between 3 and 12 months. Analysis of covariance was used to compare SA-BMC at 3 and 12 months and within individuals.

RESULTS

Infant characteristics

In all, 30 infants (13 males and 17 females) were studied at mean \pm s.d. 91 ± 6 and 366 ± 6 days after delivery. All were reported to be well during both sampling periods. No maternal health problems (for example, mastitis) likely to affect breast-milk intake were reported.

Weight, length, BMC, BW and SA-BMC increased significantly during 3–12 months. However, weight and length s.d.-scores became more negative, indicating growth faltering (Table 1).

Breast-milk intake

At 3 months, 9 (30%) infants were exclusively breast-fed, 13 (43%) were breast-fed and occasionally given small amounts of water, and 8 (27%) were breast-fed and given complementary foods and water. At 12 months, all infants were breast-fed and given other foods and water. Mean \pm s.d. breast-milk intake was 0.875 ± 0.198 and 0.763 ± 0.213 l per day at 3 and 12 months, respectively (Table 2). There was a considerable variation between infants; IQR was 0.734–1.049 and 0.607–0.898 l per day at 3 and 12 months,

Table 1. Characteristics of the 30 infants at 3 and 12 months of age^a

	3 months	12 months	P-value
Age (days)	91 ± 6	366 ± 6	≤ 0.0001
Weight (kg)	5.74 ± 0.76	8.00 ± 0.95	≤ 0.0001
Length (m)	0.593 ± 0.024	0.710 ± 0.030	≤ 0.0001
BMC (g/cm)	0.110 ± 0.018	0.138 ± 0.027	≤ 0.0001
BW (cm)	0.518 ± 0.072	0.669 ± 0.071	≤ 0.0001
Weight s.d.-score ^b	-0.441 ± 1.07	-1.967 ± 1.06	≤ 0.0001
Weight s.d.-score ^c	-0.512 ± 1.01	-1.310 ± 0.92	≤ 0.0001
Length s.d.-score ^b	-0.511 ± 1.04	-1.469 ± 1.13	≤ 0.0001
Length s.d.-score ^c	-0.592 ± 1.03	-1.512 ± 1.13	≤ 0.0001

Abbreviations: BMC, bone mineral content; BW, bone width. ^aData are presented as mean \pm s.d. ^bs.d. score calculated using the 1990 British Growth Reference.¹⁷ ^cs.d.-score calculated using the WHO Standards.¹⁸

Table 2. Sources of calcium intake of the 30 infants at 3 and 12 months of age

	3 months			12 months		
	Mean \pm s.d.	Median	IQR	Mean \pm s.d.	Median	IQR
Breast-milk intake (l/day)	0.875 \pm 0.198	0.848	0.734, 1.049	0.763 \pm 0.213	0.783	0.607, 0.898
Breast-milk calcium concentration (mg/l) ^a	203 \pm 27	204	185, 215	152 \pm 24	149	135, 165
Calcium intake from breast milk (mg/day) ^a	179 \pm 53	182	133, 203	117 \pm 38	121	79, 152
Calcium intake from other sources (mg/day) ^{a,b}	12 \pm 38	0	0, 4	73 \pm 105	36	23, 85
Total calcium intake (mg/day) ^a	191 \pm 59	190	146, 220	190 \pm 95	178	140, 205

Abbreviation: IQR, interquartile range. ^aTo convert mg to mmol of calcium, divide by 40. ^bEight children received solids at 3 months of age (mean 219, median 235, IQR 172, 260).

Table 3. Comparison of males and females

	3 months			12 months		
	Males (n = 13)	Females (n = 17)	P-value	Males (n = 13)	Females (n = 17)	P-value
	Mean \pm s.d.	Mean \pm s.d.		Mean \pm s.d.	Mean \pm s.d.	
Age (days)	91.6 \pm 4.7	91.3 \pm 8.1	0.9	366.1 \pm 7.8	365.8 \pm 4.5	0.9
Weight (kg)	6.15 \pm 0.63	5.42 \pm 0.74	0.004	8.67 \pm 0.86	7.58 \pm 0.81	0.004
Length (mm)	605 \pm 22	586 \pm 21	0.025	728 \pm 33	701 \pm 25	0.039
Breast-milk intake (g)	948 \pm 175	819 \pm 201	0.08	763 \pm 237	762 \pm 200	1.0
Breast-milk [Ca] (mg/l) ^a	20.3 \pm 2.4	20.3 \pm 3.0	1.0	15.4 \pm 2.0	15.1 \pm 2.7	0.7
Breast-milk Ca intake (mg/day) ^a	193 \pm 46	168 \pm 56	0.2	117 \pm 39	116 \pm 39	0.9
BMC (g/cm) ^b	0.114 \pm 0.015	0.106 \pm 0.020	0.2	0.141 \pm 0.023	0.135 \pm 0.030	0.6
BW (cm)	0.522 \pm 0.062	0.514 \pm 0.081	0.8	0.682 \pm 0.053	0.660 \pm 0.082	0.4
Total Ca intake (mg/day) ^a	205 \pm 60	180 \pm 56	0.2	208 \pm 123	177 \pm 67	0.4

Abbreviations: BMC, bone mineral content; BW, bone width; Ca, Calcium; [Ca], calcium concentration. ^aTo convert mg to mmol of calcium, divide by 40.

^bThere were no significant differences in size-adjusted bone mineral content (SA-BMC) between males and females at 3 and 12 months ($P = 0.22$, $P = 0.16$).

respectively. Breast-milk intake was consistent within individuals ($P = 0.005$) and decreased between 3 and 12 months ($P = 0.008$).

Breast-milk calcium concentration

Mean \pm s.d. breast-milk calcium concentration was 203 \pm 27 and 152 \pm 24 mg/l at 3 and 12 months, respectively (Table 2). The concentration varied between women; the IQR was 185–215 and 135–165 mg/l at 3 and 12 months, respectively, but was consistent within individuals ($P = 0.0003$) and decreased between 3 and 12 months ($P \leq 0.0001$).

Breast-milk calcium intake

Mean \pm s.d. calcium intake from breast milk was 179 \pm 53 and 117 \pm 38 mg/day at 3 and 12 months, respectively (Table 2). There was no relationship between breast-milk intake and breast-milk calcium concentration at 3 ($P = 0.08$) or 12 months ($P = 0.31$). There was a considerable variation in breast-milk calcium intake between infants; the IQR was 133–203 and 79–152 mg/day at 3 and 12 months, respectively. Intake was consistent within individuals ($P = 0.0008$), and decreased between 3 and 12 months ($P \leq 0.0001$).

Complementary food intake

Median calcium intake from complementary foods was 0 (IQR 0, 4) and 36 (IQR 23, 85) mg/day at 3 and 12 months, respectively ($P = 0.003$) (Table 2).

At 3 months, eight infants (27%) received complementary foods; two on 2 days, two on 3 days and four on 5 days. These were thin porridges made from cereal flour (generally rice or millet), water, salt and occasionally some sugar. The weight of porridge on days it was consumed was 226 \pm 40 g/day. Over 5

days, the mean \pm s.d. weight consumed was 164 \pm 54 g/day, representing 8 \pm 3 mg Ca/day. Two infants (7%) also had fresh or sour cow's milk separately or added to porridge on 2 or 3 days. The mean weights consumed were 146 and 199 g/day, representing 58 and 119 g/day over 5 days, contributing 74 and 152 mg/day to the calcium intake.

At 12 months, all the infants received complementary foods: one on 1 day, one on 2 days, eight on 4 days and 20 every day. The foods were porridge (as above) and adult foods (steamed rice, millet and sorghum, with sauces made mainly from groundnuts or leaves). Ingredients rich in calcium (most commonly dried fish and dried baobab leaves) were present in 44% of the sauces. Five infants (17%) had fresh cow's milk separately or added to foods: three on 1 day, one on 3 days and one on 5 days. On days when milk was consumed, mean \pm s.d. weight was 312 \pm 105 g/day. Averaged over 5 days, this represented an intake of 151 g/day, contributing 193 mg/day to calcium intake.

Total calcium intake

Mean \pm s.d. total calcium intake was 191 \pm 59 and 190 \pm 95 mg/day at 3 and 12 months, respectively. There was no difference between 3 and 12 months. At both ages, at least 75% of infants had a total calcium intake < 200 mg/day. Breast milk was the predominant source of calcium, contributing 94% and 62% to the total intake at 3 and 12 months, respectively (Table 2).

Comparison of male and female infants

Males were heavier ($P = 0.004$, $P = 0.004$) and longer ($P = 0.025$, $P = 0.039$) than females at 3 and 12 months, but there were no differences in BW or BMC before or after adjusting for bone and body size. There were no differences in breast-milk intake, breast-milk

calcium concentration, breast-milk calcium intake or total calcium intake between males and females at 3 or 12 months (Table 3).

Calcium intake as a predictor of infant growth and bone mineral accretion

At 3 months, breast-milk calcium intake positively predicted weight ($R^2 = 39\%$, $P = 0.0002$) and length ($R^2 = 13\%$, $P = 0.056$) but not BMC, BW or SA-BMC ($P > 0.4$). When breast-milk intake and breast-milk calcium concentration were included as independent variables in multiple regression models rather than combined as breast-milk calcium intake, breast-milk intake significantly predicted weight ($R^2 = 46\%$, $P \leq 0.0001$) and marginally length ($R^2 = 13\%$, $P = 0.056$), but breast-milk calcium concentration did not ($P > 0.1$). These relationships were similar when adjusted for sex. At 3 months, insufficient infants received foods to investigate the relationships of total calcium intake with growth and bone variables. There were no differences between infants exclusively breast-fed, breast-fed plus water or breast-fed plus complementary food, in weight, length, BMC or BW in models adjusted for sex ($P > 0.1$).

At 12 months, breast-milk calcium intake positively predicted weight ($R^2 = 47\%$, $P \leq 0.0001$) and length ($R^2 = 32\%$, $P = 0.001$) but not BMC, BW or SA-BMC ($P > 0.6$). There were no relationships between weight, length, BMC, BW or SA-BMC and total calcium intake ($P > 0.4$) or calcium intake from other foods ($P > 0.5$). However, in univariate models breast-milk intake positively predicted weight ($R^2 = 39\%$, $P = 0.0002$) and length ($R^2 = 26\%$, $P = 0.004$), as did breast-milk calcium concentration ($R^2 = 19\%$, $P = 0.015$; $R^2 = 15\%$, $P = 0.038$). In multiple regression models with each component of calcium intake included, breast-milk intake was the most significant predictor of weight and length ($P = 0.0004$ and $P = 0.004$, respectively). Breast-milk calcium concentration remained significant only for weight ($P = 0.024$). The relationships were similar when adjusted for sex. There were no significant correlations between breast-milk intake, breast-milk calcium concentration or calcium intake from other foods with BMC, BW or SA-BMC.

DISCUSSION

As far as we are aware, this is the first study to fully quantify total calcium intake from breast milk and complementary foods of individual infants in a community with habitually low calcium intakes and prolonged breast-feeding. At 3 months, 30% of infants were exclusively breast-fed, 43% had breast milk and water, and 27% had breast milk, water and complementary foods. At 12 months, all infants were breast-fed and received complementary foods.

The study size was limited by the intense protocol. However, we feel confident that the feeding patterns were broadly representative of this rural area of Africa because of the universal practice of prolonged breast-feeding and using complementary foods of limited quality and variety.

The average total calcium intake was < 200 mg/day. Breast milk contributed 94% at 3 months and, despite the introduction of adult-style foods, provided the majority of calcium intake (62%) at 12 months. The contribution of cow's milk was low; only two infants consumed it at 3 months and only five occasionally at 12 months. This indicates the importance of breast milk as a major source of calcium when exclusive breast-feeding has ceased in regions where complementary and adult foods have low calcium density. The calcium intakes were much lower than those in western countries. For example, in the United Kingdom, average intakes after exclusive breast-feeding ceases are 600–700 mg/day.¹⁹

Breast-milk calcium concentration at 3 months was 203 mg/l; 34% lower than in British women (307 mg/l).⁹ A low breast-milk calcium concentration has been reported among some

populations of women in Africa and elsewhere. The reasons are unknown. Our calcium supplementation studies in The Gambia have shown that concentration is unrelated to contemporaneous maternal calcium intake, or that in the preceding pregnancy, and that pre- and post-natal growth and bone mineral accretion in infants is not influenced by maternal calcium supplementation during pregnancy or lactation.^{10,11}

All the infants in this community are breast-fed on demand, and sleep with their mothers, so have unlimited access to the breast. Breast-milk intake was 0.875 l per day at 3 months. Similar intakes have been reported at this age in developed and developing countries.^{12,20,21} At 12 months, breast-milk intake was 0.763 l per day, lower than at 3 months ($P = 0.008$), but high compared with western populations measured during extended lactation.²² This probably partly reflects the traditions of 2 years' demand breast-feeding and of infants sleeping with their mothers, and may also reflect the greater reliance placed on breast milk as the main source of nutrition for infants and young children.

Total calcium intake at 3 months (190 mg/day) was very close to the theoretical calcium accretion rate in infancy²³ and to the Adequate Intake described by the US Institute of Medicine for breast-fed infants aged 0–6 months.²⁴ However, intake at 12 months (191 mg Ca/day) was considerably lower than the Estimated Average Requirement (500 mg Ca/day) and Recommended Dietary Allowance (700 mg Ca/day) set by the Institute of Medicine for children aged 1–3 years. Similarly, the intake was substantially lower than the UK Reference Nutrient Intake (525 mg/day) for infants aged 0–12 months.²⁵ This partially reflects the methods of calculating dietary reference values at this age because no allowance is made for the higher absorption of calcium from breast milk. They are therefore not designed for young children who continue to receive substantial quantities of breast milk.

Growth faltering and stunting are common features of infancy in many developing countries, including The Gambia.^{20,26,27} Infants had lower weight and length s.d.-scores at 3 months compared with both the 1990 British Growth Reference, which comprised mostly data from breast-fed infants¹⁷ and the WHO growth standards,¹⁸ which comprised data from infants exclusively breast-fed for 6 months. By 12 months, growth faltering was evident (Table 1). The average total calcium intake was close to the estimated biological requirement of 200 mg Ca/day for bone mineral accretion during infancy, supporting the possibility that the low calcium supply may limit skeletal growth. However, although weight and length were significantly related to breast-milk calcium intake at 3 and 12 months, breast-milk intake and not calcium concentration was the main variable independently related to weight and length. The exception was weight at 12 months where both breast-milk intake and breast-milk calcium concentration were positive predictors. Such relationships were also observed in our previous studies and probably reflect greater demands of a larger child. It is plausible that an infant consuming more breast milk may have better growth, but equally, one growing rapidly may consume more. There were no significant relationships between calcium intake, breast-milk intake or breast-milk calcium concentration with bone growth or mineral accretion measured by BW and BMC at the midshaft radius. This suggests that the main limitation on linear growth or bone mineral accretion is not calcium intake, although this conclusion is limited by the size of the study. It is possible that the infants' intake of other nutrients may be limiting or that there are other factors such as recurrent infections that are affecting linear growth. Although we did not measure the energy or protein content of breast milk in this study, our previous work in this community has shown that women of marginal nutritional status are able to produce milk of similar macronutrient composition to those who are well-nourished, and only under conditions of severe undernutrition is

milk macronutrient quality compromised.^{19,20,28,29} However, dietary energy restriction and vitamin and mineral deficiencies are known to be common in this community.^{29,30}

This study has shown that the combination of low calcium intakes from breast milk and complementary foods resulted in a total calcium intake, close to the estimated biological requirement, that did not differ between 3 and 12 months of age. However, the relationships between calcium intake and growth were largely accounted for by breast-milk intake, suggesting that a low calcium intake *per se* was not the limiting factor in the poor growth. These findings have potential implications for deriving calcium requirements for infants and young children in developing countries, but need confirmation from larger studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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