Calcium requirements of lactating Gambian mothers: effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion[1-3]

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ABSTRACT   The calcium requirement for prolonged lactation was investigated in a randomized supplementation study of Gambian mothers consuming a low-calcium diet (7.1 mmol/d, or 283 mg/d). Sixty women were studied from 10 d to 78 wk of lactation, receiving calcium or placebo for the first 12 mo. The supplement increased average calcium intake by 17.9 mmol/d (714 mg/d). Supplementation had no effect on breast-milk calcium concentration or on maternal bone mineral content. Urinary calcium output was higher in supplemented than in unsupplemented mothers by 1.18 mmol/d (47 mg/d), $P \leq 0.005$. Longitudinal changes in urinary calcium output and bone mineral content made a substantial contribution to calcium requirements for lactation. This study suggests that, in women with low calcium intakes, there is no direct benefit from increasing calcium intake during lactation, and that physiological mechanisms operate to furnish calcium for breast-milk production.   *Am J Clin Nutr* 1995;62:58–67.

KEY WORDS   Calcium requirement, calcium supplementation, lactation, breast milk, bone mineral content, urinary excretion

INTRODUCTION

Breast-feeding mothers secrete ≈5 mmol (200 mg) Ca into breast milk every day (1). To ensure that this extra requirement is met, recommended calcium intakes for women are higher during lactation. Advice varies, but most recommended dietary allowances (RDAs) are ≈30 mmol (1200 mg) Ca/d (1). Current recommendations for lactating women by the FAO/WHO, the United States, and the United Kingdom are 25–30, 30, and 31.25 mmol/d (1000–1200, 1200, and 1250 mg/d), respectively, representing an increase of 10–15 mmol/d (400–600 mg/d) relative to nonpregnant, nonlactating (NPNL) women (1).

The consequences of low calcium intake during lactation are not known (1). In principle, inadequate calcium supply may reduce breast-milk calcium secretion, with effects on the growth and bone development of the breast-fed baby. Alternatively, breast-milk production may be subsidized by calcium released from the maternal skeleton, possibly increasing the mother's fracture risk during lactation or later in life. However, it is also possible that increases in calcium retention, caused by enhanced absorption and/or decreased excretion, may furnish sufficient calcium for breast-milk production without the need for changes in dietary calcium supply.

Mandinka women in rural areas of The Gambia, West Africa, in common with many women elsewhere in the developing world, consume a diet that is low in calcium, averaging ≈10 mmol Ca/d (400 mg/d), and have little opportunity to increase calcium intake during lactation (2). In addition, their calcium requirement remains high for many years because of numerous pregnancies and long lactation periods.

Observational studies among Mandinka women have shown that the concentration and daily output of calcium in their breast milk are significantly lower than those of British mothers (3), and that the ratio of calcium to phosphorus in their breast milk is unusually low (4). Although osteoporosis is not a clinical problem for elderly Gambian women (5), absorptiometric studies have demonstrated that the peak bone mass of rural Gambian women is not markedly different from that of British women, before or after adjustment for body size (5, 6). In addition, they experience substantial bone loss in the forearm, lumbar spine, and femoral neck in middle life, leading to low bone mineral contents (BMCs) in old age (5, 6). This suggests that some as yet unidentified facets of Gambian lifestyle or physiology may be responsible for the low incidence of fragility fractures rather than superiority of bone mineral mass (5, 6). The bone findings were unexpected because American blacks, who largely originate from West Africa, are known to have a greater BMC than whites (5). A possible explanation is that low calcium intake coupled with long periods of elevated calcium requirement may adversely affect bone mineral mass in Gambian women.

These studies, therefore, suggested that the low calcium intake of rural Gambian women may not be adequate for optimal breast-milk calcium secretion or for optimal development of peak bone mass. The aim of this study was to determine whether lactating Gambian mothers would benefit by an increase in calcium intake to a value close to that recommended by the FAO/WHO for breast-feeding women. The investigation took the form of a randomized, double-blind, placebo-controlled intervention study in which a calcium supplement was provided for the first 12 mo of lactation. The effects on breast-milk calcium concentration, maternal BMC, and urinary calcium excretion were measured longitudinally throughout 18 mo of lactation.

SUBJECTS AND METHODS

Subjects

Sixty women aged 16–41 y (parity 1–13), from the rural villages of Keneba and Manduar, West Kiang, The Gambia, participated in the study. These villages form part of the survey area of the MRC Dunn Nutrition Unit and have been the focus of detailed nutritional, demographic, and medical studies for many years. All mothers in this region breast-feed their infants, fully and on demand, for ≈2 y, with weaning foods introduced from ≈3 mo.

The study was approved by the MRC Gambia Ethics Committee. Consent was obtained after the subjects, who were illiterate, had been given a full verbal description of the study in their own language. It was emphasized that participation was voluntary and participants could withdraw at any time.

All healthy women giving birth in the two villages between March 1990 and March 1991 were invited to join the study. Mothers with twins were excluded. All eligible women agreed to participate and there were no withdrawals, except for one mother whose infant died at 3 mo. Her data were omitted from further consideration and an extra subject was recruited.

Supplementation

The subjects were randomly assigned, double-blind, to receive either a calcium supplement or a placebo for 12 mo. The calcium supplement consisted of 25 mmol (1000 mg) Ca as the carbonate, taken 5 d each week as two orange-flavored, chewable tablets (Calcichew; Shire Pharmaceuticals Ltd, Hampshire, UK). The placebo tablets were orange-flavored dextrose (Dextro-energy; CPC UK Ltd, Surrey, UK). The calcium and placebo tablets were similar in taste and texture but differed in shape. The identity of the tablets was not known by any subject or investigator in The Gambia. On testing, the calcium tablets dissolved in dilute acid (0.3 mol HCl/L, room temperature, 5–10 min), indicating that this brand of calcium carbonate is likely to dissolve readily in vivo (7).

The subjects were assigned, in sequence, a study number at the first measurement session. For each study number there was a corresponding labeled plastic jar prepacked with sufficient tablets of either supplement or placebo for the 12-mo supplementation period. A randomization procedure based on permuted blocks of four was used to assign study numbers to treatment groups, to minimize any confounding effects of season on study outcome. Group assignment and jar preparation were done in Cambridge by a colleague who was not otherwise

involved in the study. The randomization code was not made available to any member of the research team, either in Cambridge or The Gambia, until all the fieldwork was completed and the primary data entry and laboratory analyses were performed.

The period of supplementation began the day after the baseline measurements were completed, including the 24-h urine collection (see below), and ended 52 wk later, after the measurements and sample collections at this time point were completed. The tablets were consumed in the early evening (between 1700 and 1900) between the two main meals of the day, which were eaten midafternoon and evening. The supplement was not ingested with meals to minimize any possible adverse effects on the absorption of other minerals (8, 9). This was of particular concern in this study because iron deficiency is common among Gambian women.

Fieldworkers delivered the tablets personally to each subject daily and observed their consumption. Any tablets not taken because of illness or absence from the village were taken on weekends. In the fasting month of Ramadan, the tablets were delivered and consumed later in the evening, after the subjects had broken fast but before the main meal. Compliance was 100% and the tablets were well-accepted. There were no complaints of adverse side effects.

Data collection

Each subject visited the Dunn Nutrition Unit's Keneba clinic for baseline measurements during the second week after delivery, on the day after their traditional confinement period ended ($\bar{x} \pm$ SD: 9 $\pm$ 1 d postpartum). At this clinic, the mother provided samples of breast milk and fasting blood, a 24-h urine collection was started, and anthropometry and single-photon absorptiometry were performed. These measurements were repeated at 13, 52, and 78 wk after delivery (92 $\pm$ 6, 366 $\pm$ 5, and 547 $\pm$ 5 d postpartum, respectively). Additional breast-milk samples were collected and anthropometry performed at 6, 19, 26, 39, and 65 wk (42 $\pm$ 1, 134 $\pm$ 2, 182 $\pm$ 1, 273 $\pm$ 1, and 455 $\pm$ 2 d postpartum, respectively). Dietary intake estimates were performed at the 13- and 52-wk time points.

All but one subject were still breast-feeding at 78 wk postpartum. This mother had weaned her baby after becoming pregnant. Subsequent investigation indicated that two other mothers had been in the early stages of pregnancy at 78 wk. The 78-wk data from these three women were excluded from data analysis.

Breast-milk analysis

At each sample collection, breast milk (1–2 mL) was expressed manually from each breast separately into unused, disposable polystyrene tubes. No specific sampling protocol with respect to time of day or stage of feed was adopted, because a previous study in Keneba had demonstrated that this was unnecessary for the collection of representative milk samples for calcium analysis (3). Care was taken throughout the procedure to avoid calcium contamination. The samples were frozen immediately, transported in batches to the United Kingdom on dry ice, and stored in Cambridge at −20 °C.

Breast-milk calcium and phosphorus concentrations were measured by using a semiautomated spectrophotometric method with a centrifugal analyzer, after lyophilization of

whole-milk samples, combustion at 500 °C, and digestion with 0.3 mol HCl/L (Spectrosol, 1%; BDH Chemicals, Poole, UK) (10). The standards were diluted to contain the same concentration of acid as the samples. The procedure was performed in duplicate for all samples. Accuracy and precision were monitored by including reference material (Reference Bovine Milk Powder SRM 1549; National Bureau of Standards, Gaithersburg, MD), and an aliquot from a pooled breast-milk sample, with each batch of assays. Average results for samples of breast milk collected from a subject's left and right breasts were computed at each time point and used in subsequent data analysis.

Bone mineral measurements

The BMC (g/cm) and scanned bone width (BW, cm) of the radius were measured in The Gambia with a Norland 2780 single-photon absorptiometer (Fort Atkinson, WI). Measurements were made at two skeletal sites: *1*) midshaft radius, a predominantly cortical bone site located at the level on the forearm corresponding to two-thirds distance along the ulna between the olecranon and styloid process in the distal direction; and *2*) radial wrist, which contains a higher proportion of trabecular bone, measured at the position where the intraosseous space between the ulna and radius is 5 mm.

Calibration of the instrument was performed at the start of each measurement session. Between-day precision, assessed by regular scanning of phantom reference bone material containing 0.54 and 1.52 g/cm, was 1.3% and 1.0%, respectively. The reproducibility of forearm absorptiometry judged by repeat measurements of individuals is 2–5%. Single-photon absorptiometry measures the attenuation of a beam of ^{125}I radiation as it is scanned across the region of interest (11). The effective dose received by a premenopausal woman during one complete set of shaft and wrist scans (0.1 μSv) is extremely low and is within background values.

Successful bone mineral measurements were obtained from all Gambian subjects at baseline and at 13 and 52 wk. However, because of instrument malfunction in the later stages of the study, bone mineral measurements were only obtained for 25 subjects at 78 wk.

Urine outputs

Urine was collected over a 24-h period in the subject's home or fields, as necessary. All containers and apparatus were acid-washed to minimize calcium contamination. Because of the high ambient temperatures in The Gambia and the lack of electricity in the villages, the subjects were provided with a urine collection kit comprising urine bottles, a funnel, and a cooler containing frozen cold packs. A fieldworker attended the subject at the start and end of the collection period. In addition, the fieldworker visited the subject at regular intervals during the day, refreshing the supply of cold packs and returning filled urine bottles to the laboratory refrigerator.

In the laboratory, the urine fractions were pooled and mixed, and the total volume recorded. Aliquots were taken for storage and for the measurement of pH and titratable acidity (obtained by direct titration to pH 7.4). The aliquot for the analysis of calcium, phosphate, and creatinine was acidified with concentrated HCl to a final acid concentration of 0.3 mol/L (1%). The samples were stored at −20 °C and transported to the United

Kingdom on dry ice. A pilot study demonstrated that this protocol, which removed the necessity of sending urine bottles containing concentrated acid into the villagers' homes, was effective in preventing the precipitation of calcium-containing deposits during storage (A Prentice, unpublished data, 1989).

The urine samples were assayed for calcium, phosphate, and creatinine by using commercial kits on a centrifugal analyzer (Roche Products, Welwyn Garden City, UK). The analytical methods for calcium were based on methylthymol blue reaction in the presence of 8-hydroxyquinoline, those for phosphate were based on direct phosphomolybdate reaction without deproteinization, and those for creatinine were based on kinetic buffered Jaffé reaction without deproteinization. In common with the breast-milk assay, the calcium standard for urinalysis was diluted to the same acid concentration as in the samples (10). In addition, creatinine was determined in a fasting plasma sample, collected between 0800 and 1000 before the start of the urine collection, and was anticoagulated with lithium heparin for the calculation of creatinine clearance rate. All assays were performed in duplicate. Quality-assurance materials (Lyphocheck Normal Urine Control, Bio-Rad, Anaheim, CA; Roche Control Serum N, Roche Products, Welwyn Garden City, UK) were included with all batches of samples to monitor accuracy and precision.

The creatinine clearance rate was used to evaluate the likely completeness of urine collection. A calculated creatinine clearance rate of < 60 mL/min per 1.73 m^2 was obtained for 2, 4, and 12 subjects at 13, 52, and 78 wk respectively, possibly indicating incomplete collection. Urine collections with a creatinine clearance rate below this arbitrary threshold were excluded from subsequent analysis and data presentation. This had little effect on absolute urine results or those expressed relative to creatinine, except at 78 wk. The interpretation of results was unaltered if the more conservative cutoff of 70 mL/min per 1.73 m^2 was used.

Dietary intake measurements

Intakes of calcium and other nutrients were estimated over 5 consecutive days at 13 and 52 wk postpartum, by using a direct-weighing method (2). Each subject was visited by a fieldworker several times a day during the measurement period. At mealtimes, all items consumed and any leftovers were weighed and recipes for all dishes obtained. Details of any snacks consumed since the previous meal were recorded. Compositional information was obtained from the comprehensive analytical database of Gambian foods at the Dunn Nutrition Unit (2). These data had been obtained by analyzing raw ingredients and cooked dishes, taking account of potentially hidden sources of calcium, such as fish bones. Records of water consumption were not kept because water in the region contains little calcium (< 0.25 mmol/L, or < 1 mg/dL) (2).

Comparative data

Comparative, contemporaneous data were obtained from 15 lactating British women living in Norwich, Norfolk, UK. These women were 22–41 y old, had a parity of 1–4, and gave informed written consent to take part in the study. The mothers were studied at one time point only, 13 wk postpartum ($\bar{x} \pm$ SD: 88 ± 8 d). Techniques identical to those used in the Gambian study were used for the collection and assay of breast

TABLE 1
Characteristics of the Gambian subjects[1]

	Total (n = 60)	Supplement group (n = 30)	Placebo group (n = 30)
Age (y)	28 ± 8	28 ± 7	28 ± 8
Parity	5 ± 3	5 ± 3	6 ± 3
Weight at 1.5 wk (kg)	54.9 ± 7.5	53.9 ± 5.7	55.9 ± 8.9
Height (m)	1.59 ± 0.05	1.59 ± 0.05	1.60 ± 0.06
Infant weight at 1.5 wk (kg)	3.19 ± 0.46	3.19 ± 0.48	3.20 ± 0.45
Infant weight at 52 wk (kg)	8.11 ± 0.91	8.26 ± 0.90	7.97 ± 0.93

[1] $\bar{x}$ ± SD.

milk and urine. Bone measurements were made with a Lunar (Madison, WI) SP2 absorptiometer at the same skeletal sites as for the women in The Gambia. Cross-calibration of the two instruments was made by using phantom materials. Dietary information was obtained by direct weighing over 7 d. The Norwich study was approved by the Ethics Committee of the Institute of Food Research.

In addition, comparisons were made with urine data obtained in connection with a separate study, from seven NPNL Gambian women living in the same villages and with the same age range as the lactating mothers (TJ Aspray, A Prentice, unpublished data, 1995).

Statistical analysis

Data were analyzed by using a combination of analysis of variance, regression analysis, and analysis of covariance with LINEAR MODEL software on DataDesk 4.1 (Data Description Inc, Ithaca, NY). Scheffé post hoc tests were used to test the significance of differences between groups of data. Where appropriate, data were transformed to natural logarithms to correct for skewed distributions and to permit exploration of power and proportional relations (12). In natural logarithms, group differences × 100 correspond closely to percentage differences calculated as (difference/mean) x 100 (13). For consistency, all percentages quoted in the text were calculated in this manner.

Where appropriate, BMC at either site on the forearm was corrected for BW and body size by using a multiple-regression approach to prevent size-related artifacts arising from the derivation of areal bone mineral density (BMD) (12). Adjustment

for possible seasonal effects in statistical models was made by dividing each year into three seasons: wet (July–October), harvest (November–February), and hot (March–June) (2). Exploration of group effects on time trends within individuals was performed by multiway analysis of variance with subjects nested by group, a method of repeated-measures analysis suitable for use with unbalanced data sets.

The primary outcome variables for the supplementation study were mature breast-milk calcium concentration and change in bone mineral status. Typical population CVs for these factors are 14% for breast-milk calcium concentration adjusted for stage of lactation, and 3% and 8% for longitudinal measurements of BMC at the radius shaft and wrist, respectively, using the Norland instrument (3, 5; A Prentice, unpublished data, 1995). The study, therefore, had the statistical power (5% significance and 80% power) to detect differences between supplement and placebo groups of 10%, 2%, and 6%, respectively.

RESULTS

Characteristics of the subjects

There were no differences between Gambian mothers in the supplemented and placebo groups in age, parity, height, weight at baseline, and growth of their infants (**Table 1**). The mean (± SD) characteristics of the comparative group of 15 British women at 13 wk of lactation were as follows: age = 29 ± 5 y, parity = 2 ± 1, weight = 64.6 ± 11.0 kg, and height = 1.64 ± 0.06 m.

Intake of calcium and other nutrients

The dietary intakes of the Gambian subjects at 13 and 52 wk and those of the British women at 13 wk are given in **Table 2**. There were no significant differences in calcium intake from the diet between supplemented and Gambian unsupplemented mothers at either time point, nor in the intakes of the other nutrients quantified.

Analysis of variance showed that, after adjustment for season, calcium intake measured on two occasions 9 mo apart was characteristic of the individual but was not influenced by lactation stage ($P \leq 0.001$). Similar analyses for phosphorus, energy, protein, and fat showed that, after adjustment for season, intakes of these nutrients were characteristic of the

TABLE 2
Dietary intakes of Gambian and British lactating mothers at 13 and 52 wk of lactation[1]

	The Gambia				
	13 wk		52 wk		Britain, 13 wk (n = 15)
	Supplement group (n = 30)	Placebo group (n = 30)	Supplement group (n = 30)	Placebo group (n = 30)	
Calcium (mmol/d)	6.88 ± 2.85[2]	7.20 ± 3.20	7.15 ± 3.15[2]	7.13 ± 2.88	29.20 ± 7.90
Phosphorus (mmol/d)	26.0 ± 8.0	26.7 ± 8.3	23.8 ± 6.9	23.8 ± 8.1	47.9 ± 14.5
Energy (MJ/d)	8.07 ± 2.27	7.78 ± 2.06	6.84 ± 1.60	6.53 ± 1.77	10.02 ± 2.62
Protein (g/d)	57.8 ± 15.9	59.2 ± 16.6	51.3 ± 12.4	49.5 ± 15.0	81.4 ± 26.5
Fat (g/d)	54.7 ± 25.9	51.8 ± 24.4	38.3 ± 15.5	36.2 ± 18.0	100.3 ± 37.3

[1] $\bar{x}$ ± SD. Gambian data were collected longitudinally; British data were collected from women in Norwich, UK. Multiplication factors to convert mmol/d to mg/d: calcium, 40; phosphorus, 31.

[2] Excludes the calcium supplement at 17.9 mmol/d.

individual ($P \leq 0.001$) and were significantly lower at 52 wk than at 13 wk of lactation ($P \leq 0.001$). The changes in intake from 13 to 52 wk were as follows: phosphorus, -14%; energy, -18%; protein, -18%; fat, -38%. No differences in intake emerged between the supplement and placebo groups after adjustment for time point, season, and individual.

Overall, the dietary calcium intakes of the Gambian mothers were low, with a mean ($\pm$ SD) of 7.1 $\pm$ 3.0 mmol/d (283 $\pm$ 120 mg/d). The calcium-to-phosphorus ratio was also low at 0.26 $\pm$ 0.08 (mmol/mmol), or 0.34 $\pm$ 0.10 (mg/mg). This contrasts with the comparative group of 15 British lactating mothers (Table 2) who had a calcium intake of 29.2 $\pm$ 7.9 mmol/d (1168 $\pm$ 316 mg/d) and a calcium-to-phosphorus ratio of 0.64 $\pm$ 0.09 (mmol/mmol), or 0.82 $\pm$ 0.12 (mg/mg).

The calcium supplement provided an extra 17.9 mmol/d (714 mg/d) averaged over the 52-wk period. This extra represented 0.33 mmol $\cdot$ kg^{-1} $\cdot$ d^{-1} (13.0 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}) expressed relative to body weight. The mean ($\pm$ SD) total calcium intake of the supplemented mothers during the supplementation period was 24.9 $\pm$ 3.0 mmol/d (995 $\pm$ 120 mg/d), more than three times higher than the intake of women in the placebo group ($P \leq 0.001$).

Breast-milk calcium concentration

No significant differences were observed in breast-milk calcium concentration between the supplement and placebo groups at any time point from 6 to 78 wk of lactation (**Figure 1**). In addition, there were no significant differences in breast-milk phosphorus concentration or the ratio of calcium to phosphorus.

The mean calcium and phosphorus concentrations in mature Gambian breast milk at each time point, plus the ratio of calcium to phosphorus, are given in **Table 3** for the supplemented and placebo groups combined. Analysis of variance showed that breast-milk calcium concentration decreased within individuals as lactation progressed ($P \leq 0.001$; change from 6 to 78 wk = -40%), and was characteristic of the individual after correction for stage of lactation ($P \leq 0.001$). No relations were found between breast-milk calcium concen-
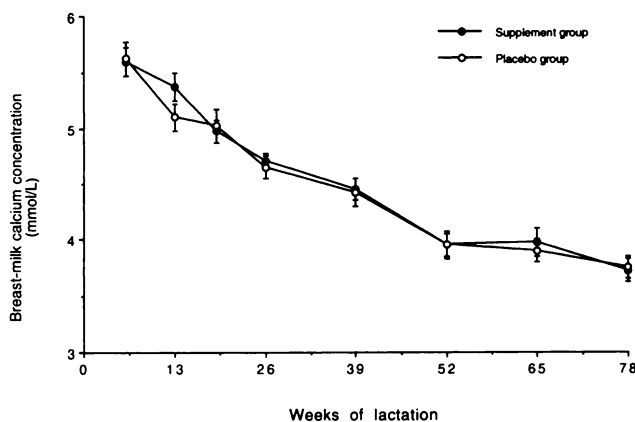
tration, adjusted for stage of lactation, and age, parity, season, or calcium intake (with or without the calcium supplement).

By chance, there was a significant difference in calcium concentration between supplement and placebo groups in the sample of transitional milk collected at baseline ($\bar{x} \pm$ SD: supplement, 5.15 $\pm$ 1.25 mmol/L; placebo, 5.98 $\pm$ 1.33 mmol/L; $P = 0.015$). A preliminary analysis indicated a significant difference between groups in the mean change in breast-milk calcium concentration between baseline and 6 wk, suggesting a positive effect of the supplement (supplement, +0.48 mmol/L; placebo, -0.42 mmol/L; $P = 0.003$). However, this calculation assumed that the change was independent of the initial value, which is rarely the case becauses extreme values tend to regress toward the mean. In this data set, regression analysis showed that such an assumption was not valid because the apparent effect of the supplement disappeared when the change in concentration was adjusted for baseline value ($P = 0.11$, NS). Overall, the mean ($\pm$ SD) concentrations in Gambian transitional milk were as follows: calcium, 5.58 $\pm$ 1.35 mmol/L (22.3 $\pm$ 5.4 mg/dL); and phosphorus, 6.32 $\pm$ 1.19 mmol/L (19.6 $\pm$ 3.7 mg/dL). The calcium-to-phosphorus ratio was 0.88 $\pm$ 0.16 (mmol/mmol), or 1.14 $\pm$ 0.21 (mg/mg).

The breast-milk calcium concentration of Gambian mothers at 13 wk of lactation was significantly lower than that of the comparative group of 15 British mothers (Gambian compared with British milk: -22%, Table 3). Because there was no difference in phosphorus concentration, there was a significantly lower calcium-to-phosphorus ratio in the Gambian milk (Table 3).

Bone mineral status

No significant differences in BMC at the radius shaft and wrist were observed between mothers in the supplement and placebo groups at any stage of lactation (**Figure 2**).

The mean BMC and BW values at 1.5, 13, and 52 wk of lactation are given in **Table 4** for the supplement and placebo groups combined. Analysis of variance demonstrated that, at both sites, BMC was characteristic of the individual after adjustment for lactation stage ($P \leq 0.001$), and that supplementation had no significant effect on BMC or on the pattern of change in BMC over time.

Within individuals, BMC at the midshaft radius was influenced by lactation stage ($P = 0.015$), with values at 13 wk significantly lower than at baseline and 52 wk (difference = -1.1%). No significant effect of lactation stage was observed at the wrist, although the pattern of mean BMC with time paralleled that seen at the shaft. For the 25 mothers who had successful bone measurements at 78 wk, there was no significant difference ($\bar{x} \pm$ SD) in BMC at the shaft or wrist at this time point compared with 52 wk (BMC shaft: 0.823 $\pm$ 0.072 g/cm at 52 wk, 0.832 $\pm$ 0.072 g/cm at 78 wk, change = +0.009 $\pm$ 0.027 g/cm; BMC wrist: 0.766 $\pm$ 0.094 g/cm at 52 wk, 0.782 $\pm$ 0.115 at 78 wk, change = +0.015 $\pm$ 0.097).

Although the mean body weight of the Gambian mothers was similar at 1.5, 13, 52, and 78 wk of lactation (Table 4), analysis of variance showed that when the data were adjusted for season, there was a small decrease in weight within individuals during lactation ($P = 0.013$). The difference in weight relative to baseline was -0.3%, -2.1%, and -2.7% at 13, 52, and 78 wk, respectively. Adjustment for season and weight

FIGURE 1. Effect of the calcium supplement on breast-milk calcium concentration. Values are means ($\pm$ SEMs) at different weeks of lactation for women in the supplemented and placebo groups ($n = 30$ per group). There was no significant difference between the groups at any time point.

TABLE 3
Calcium and phosphorus concentrations in mature breast milk, by week of lactation[1]

	The Gambia								Britain,
	6 wk ($n = 60$)	13 wk ($n = 60$)	19 wk ($n = 59$)	26 wk ($n = 60$)	39 wk ($n = 60$)	52 wk ($n = 60$)	65 wk ($n = 60$)	78 wk ($n = 57$)	13 wk ($n = 15$)
Calcium (mmol/L)	5.60 ± 0.78	5.23 ± 0.68	5.00 ± 0.68	4.68 ± 0.50	4.43 ± 0.63	3.98 ± 0.60	3.93 ± 0.65	3.73 ± 0.55	6.53 ± 0.95[2]
Phosphorus (mmol/L)	5.23 ± 0.71	4.87 ± 0.77	4.84 ± 0.74	4.77 ± 0.74	4.84 ± 0.74	4.87 ± 0.68	5.13 ± 0.80	4.87 ± 0.87	4.58 ± 0.61
Ca:P[3]	1.09 ± 0.16	1.09 ± 0.18	1.05 ± 0.16	1.00 ± 0.14	0.93 ± 0.17	0.82 ± 0.14	0.78 ± 0.14	0.78 ± 0.15	1.44 ± 0.23[2]

[1] $\bar{x}$ ± SD. Multiplication factors to convert mmol/L to mg/dL: calcium, 4.0; phosphorus, 3.1; Ca:P, 0.775. Gambian data were collected longitudinally from 60 mothers; values from 3 pregnant mothers were excluded at 78 wk and one sample was missing at 19 wk. British data were obtained from 15 mothers in Norwich, UK. Gambian breast milk calcium concentrations declined significantly during lactation and were characteristic of the individual ($P \leq 0.001$, *see* text).

[2] Significantly greater than Gambian value at 13 wk, $P \leq 0.001$.

[3] mmol/mmol.

changes within an individual did not alter the effects of lactation stage on BMC.

Regression analysis of the Gambian data showed that BMC was highly correlated with BW, but the relation was not one of direct proportion (power coefficient ≠ 1). At the shaft, BMC was proportional to $BW^{0.65}$, the relation changing to $BW^{0.47}$ after adjustment for weight and height. At the wrist, BMC was proportional to $BW^{0.44}$, changing to $BW^{0.32}$ after adjustment for weight and height. The effects of lactation stage and supplement group were examined by analysis of covariance after adjustment of BMC for BW or for BW, weight, and height. The interpretation of the results was the same as that with BMC alone.

Gambian shaft BMC values at 13 wk of lactation were similar to those of British women at the same lactation stage, but wrist BMC values were slightly lower ($P = 0.044$, Table 4). This difference disappeared after adjustment of BMC for BW, indicating that it was either due to differences in bone edge detection by the two absorptiometers or was a reflection of the different sizes of British and Gambian women.
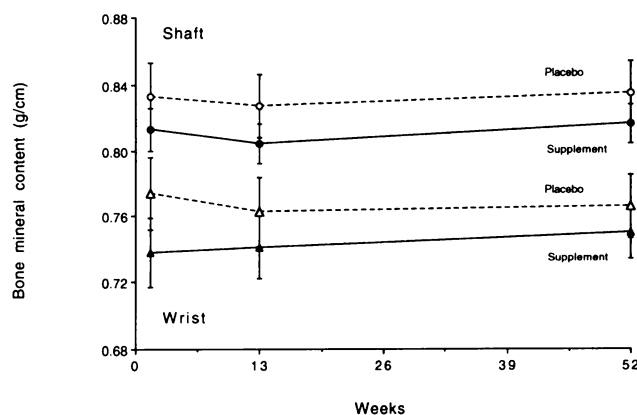

FIGURE 2. Effect of the calcium supplement on bone mineral content of the midshaft radius and radial wrist. Values are means (± SEMs) at different weeks of lactation for women in the supplemented and placebo groups ($n = 30$ per group). There was no significant difference between the groups at any time point.

Urinary excretion

The daily urinary outputs of calcium, phosphorus, and creatinine are given in **Table 5** for supplement and placebo groups separately, for the comparative groups of Gambian NPNL women, and for British lactating mothers. In the placebo group, urinary calcium output changed significantly within individuals during lactation (**Figure 3**), decreasing between 1.5 and 13 wk (change = −58%) and rising in later lactation (analysis of variance: effect of stage $P = 0.014$; effect of the individual $P = 0.004$). Calcium output was similar at 1.5 and 78 wk, and was not significantly different from the Gambian NPNL women (Table 5). The calcium output of the placebo group at 13 wk was significantly lower than that of NPNL women (difference = −88%, $P \leq 0.005$). Phosphate and creatinine outputs of women in the placebo group were not affected by lactation.

In contrast with the placebo group, calcium output in the supplement group increased within individuals after 1.5 wk (change = +33%), returning toward baseline at 78 wk (analysis of variance: effect of stage $P = 0.018$; effect of the individual $P = 0.020$). Conversely, phosphate output decreased between 1.5 and 13 wk (change = −42%, analysis of variance: effect of stage $P \leq 0.001$; effect of individual, NS). Creatinine outputs did not vary significantly with lactation stage in the supplemented women.

Women in the supplement group had significantly greater calcium outputs at both 13 and 52 wk compared with women in the placebo group ($P \leq 0.005$, Table 5, Figure 3). The difference at each time point was 1.20 mmol/d (48.0 mg/d) and 1.17 mmol/d (46.5 mg/d), respectively. At these time points, supplemented women had significantly lower phosphate outputs than did women in the placebo group [3.9 mmol/d (122 mg/d) and 3.2 mmol/d (98 mg/d)]. There was no difference between the groups in calcium or phosphate outputs at 1.5 or 78 wk, or in creatinine output at any time.

Similar results were obtained when the mineral data were expressed relative to creatinine or to body weight. The mean differences between the supplemented and placebo groups at 13 and 52 wk, significant at $P \leq 0.001$, were as follows: calcium-to-creatinine ratio, 0.162 (mmol/mmol), or 57.4 (mg/g); calcium output relative to body weight, 22.6 μmol · kg^{-1} · d^{-1} (0.903 mg · kg^{-1} · d^{-1}); phosphate-to-cre-

TABLE 4
Bone mineral measurements of Gambian and British subjects, by week of lactation[1]

	The Gambia			
	1.5 wk (n = 60)	13 wk (n = 60)	52 wk (n = 60)	Britain, 13 wk (n = 15)
Radius shaft				
Bone mineral content (g/cm)	0.823 ± 0.091	0.816 ± 0.086	0.826 ± 0.086	0.826 ± 0.066
Bone width (cm)	1.17 ± 0.11	1.17 ± 0.10	1.17 ± 0.11	1.23 ± 0.07
Radius wrist				
Bone mineral content (g/cm)	0.756 ± 0.118	0.752 ± 0.108	0.758 ± 0.097	0.823 ± 0.135
Bone width (cm)	2.42 ± 0.22	2.41 ± 0.21	2.41 ± 0.19	2.34 ± 0.19
Body weight (kg)	54.9 ± 7.5	54.9 ± 8.1	53.8 ± 8.2	64.6 ± 11.0

[1] $\bar{x}$ ± SD. Shaft, site two-thirds distal from the olecranon to the styloid; wrist, site where space between ulna and radius is 5 mm. Gambian data were collected longitudinally from 60 mothers; British data were collected from women in Norwich, UK. Within individuals, Gambian radius shaft bone mineral content at 13 wk was significantly lower than at 1.5 and 52 wk (P = 0.015, see text). After adjustment for bone width, there were no significant differences in bone mineral content between Gambian and British women.

atinine ratio, 0.441 (mmol/mmol), or 121 (mg/g); phosphate output relative to body weight, 61.5 μmol · kg^{-1} · d^{-1} (1.91 mg · kg^{-1} · d^{-1}).

Titratable acid output (Table 5) was significantly lower in the supplement group at 52 wk compared with the placebo group (P = 0.03) and compared with other time points in lactation (analysis of variance: effect of lactation stage P = 0.002; effect of the individual, NS). There was no significant effect of lactation stage on titratable acidity in the placebo group. Urinary pH was not affected by supplementation at any stage of lactation. Within individuals, there was a significant effect of lactation stage on pH (Table 5) with values at 1.5 wk higher than in later lactation (analysis of variance: effect of lactation stage P ≤ 0.001; effect of the individual P ≤ 0.001).

The urinary calcium, phosphate, and creatinine outputs of the comparative group of lactating British women were substantially higher than those of any of the groups of Gambian women (P ≤ 0.005, Table 5). The results obtained for the lactating British women were within the range of values for premenopausal, NPNL British women obtained at the Dunn Nutrition Unit when similar collection procedures and analytical methods were used (data not shown). The differences between the lactating British mothers and Gambian women in the placebo group at 13 wk of lactation were as follows: calcium output, 3.97 mmol/d (159 mg/d); phosphate output, 12.7 mmol/d (391 mg/d); creatinine output, 3.0 mmol/d (339 mg/d). The marked difference in calcium and phosphate excretion between British and Gambian mothers was still evident when the data were expressed relative to creatinine output or to body weight, although the magnitude was reduced.

DISCUSSION

Mandinka women in rural parts of The Gambia, West Africa, have a diet that is based predominantly on rice, millet, groundnuts, and fish, and as a result their intake of calcium is low (2). The measured calcium intake of lactating Gambian women in this study averaged < 7.5 mmol/d (300 mg/d). This was lower than the intake of 10 mmol/d (400 mg/d) recorded in an earlier investigation (2), partly because of an increased preference for rice, rather than millet steamed with dried baobab leaf, as the main staple food (14). Even when allowance is made for the potential problem of underrecording of food consumption (2),

calcium intake in The Gambia is considerably below current recommendations for lactating women (1).

The possibility that such low intakes may be insufficient for optimal lactational performance and maternal health was suggested by previous studies that showed that Gambian women have lower breast-milk calcium concentrations than British women and do not have the superior peak bone mineral mass relative to whites that has been reported in African-Americans (3, 5). The results of this randomized, double-blind, supplementation study, however, have shown that increasing the calcium intake of lactating Gambian women threefold, to a figure close to the FAO/WHO recommendation, had no discernible effect on breast-milk calcium concentration or on maternal BMC. This suggests that, at least for women habituated to a low-calcium diet, there is no direct benefit from increasing calcium intake during the first year of lactation.

The bone measurements in this study were made at two sites on the radius by using single-photon absorptiometry. It is conceivable that calcium was retained in bone as a result of supplementation but changes in BMC went undetected, either because they were below the sensitivity of absorptiometry or because they occurred elsewhere in the skeleton. However, because the method was sufficiently sensitive to detect longitudinal changes in radial BMC of 1%, and because the two measurement sites differed in the proportion of cortical and trabecular bone, it would appear that, if there had been a bone response to calcium supplementation, it was either small or restricted to specific skeletal regions. In addition, because mothers in The Gambia generally continue lactating into the early stages of the subsequent pregnancy, this study offered no opportunity to examine the importance of calcium intakes on changes in bone mineral after breast-feeding ceases (1).

Women in the supplement group had a significantly greater urinary calcium output than women in the placebo group 3 and 12 mo after the start of supplementation. The difference averaged 1.18 mmol/d (47 mg/d), equivalent to 6.6% of the ingested dose. The effect of the calcium supplement on urinary calcium output is commensurate with that reported in other studies in which the influence of dietary change or the ingestion of calcium salts on urinary calcium output were investigated (**Table 6**). In particular, the response was similar to that predicted from detailed studies of calcium carbonate loading in healthy individuals (15, 16) and to that reported in subjects

TABLE 5
Urinary calcium and phosphate outputs of Gambian and British lactating women (by week of lactation) and of nonpregnant, nonlactating (NPNL) Gambian women[1]

	The Gambia									Britain, 13 wk (n = 15)
	1.5 wk		13 wk		52 wk		78 wk		NPNL	
	Supplement (n = 30)	Placebo (n = 30)	Supplement (n = 29)	Placebo (n = 26)	Supplement (n = 29)	Placebo (n = 27)	Supplement (n = 24)	Placebo (n = 17)	(n = 7)	
Calcium output (mmol/d)	1.31 ± 1.09	1.13 ± 0.92	1.82 ± 1.41[2]	0.62 ± 0.58	2.07 ± 1.39[2]	0.90 ± 0.72	1.55 ± 1.43	1.16 ± 1.20	1.60 ± 0.81[3]	4.59 ± 1.17[4]
Phosphate output (mmol/d)	13.5 ± 5.6	14.2 ± 4.2	8.8 ± 3.9[2]	12.7 ± 4.8	9.0 ± 4.9[2]	12.2 ± 3.1	11.6 ± 4.5	10.5 ± 4.3	12.5 ± 4.0	25.4 ± 5.2[4]
Creatinine output (mmol/d)	6.88 ± 1.76	6.93 ± 1.89	7.09 ± 1.74	7.34 ± 1.46	7.12 ± 1.42	7.32 ± 1.60	7.58 ± 2.27	6.58 ± 2.30	8.50 ± 0.90	10.34 ± 1.62[4]
Titratable acid output[5]	2.39 ± 0.89	2.37 ± 0.85	2.40 ± 0.80	2.64 ± 0.83	1.73 ± 0.77[6]	2.17 ± 0.58	2.22 ± 0.62	2.14 ± 0.56	—	—
pH	6.37 ± 0.43	6.39 ± 0.42	6.13 ± 0.32	6.08 ± 0.49	6.19 ± 0.47	6.09 ± 0.39	6.15 ± 0.33	6.24 ± 0.37	—	—

[1] $\bar{x}$ ± SD. Multiplication factors to convert mmol/d to mg/d: calcium, 40; phosphate phosphorus, 31; creatinine, 113. Collections with a creatinine clearance rate < 60 mL/min per 1.73 m² were excluded (see Methods). Gambian lactation data were collected longitudinally from 60 mothers; values from 2 women at week 13 with incomplete data and from 3 pregnant mothers at 78 wk were excluded. British data were obtained from 15 mothers in Norwich, UK. The significance of changes with stage of lactation are given in the text.

[2] Significantly different from placebo group at same time point, $P \le 0.005$.

[3] Significantly different from placebo group at 13 wk, $P \le 0.005$.

[4] Significantly different from lactating Gambian subjects at 13 wk in supplement and placebo groups and from NPNL Gambian women, $P \le 0.005$.

[5] Natural logarithms of mmol/d.

[6] Significantly different from placebo group, $P = 0.03$.

categorized as good calcium absorbers (13, 17). In addition, the supplement was associated with decreased urinary phosphate and titratable acid outputs, well-recognized effects of calcium carbonate loading (15). These urine results indicate that there are no reasons to suggest that the supplemental calcium was not available for absorption, or that the calcium physiology of the Gambian women was different from that of whites in this respect.

By the end of the 52-wk intervention period, each woman in the supplement group had consumed a total of 0.26 kg supplemental Ca. This amount is roughly equivalent to from one-quarter to one-third of whole-body calcium content. Because only 7% of the dose appeared in the urine, and because there

FIGURE 3. Effect of the calcium supplement on urinary calcium output. Values are means (± SEM) at different weeks of lactation for women in the supplemented and placebo groups (n = 30 per group). The differences between the groups at 13 and 52 wk were significant, $P \le 0.005$.

were no measurable changes in BMC or breast-milk calcium concentration attributable to the supplement, it must be concluded that net fractional calcium absorption was reduced substantially in the supplemented women, due either to decreased absorption efficiency and/or to increased calcium excretion into the gastrointestinal tract.

It is noteworthy that the urinary response to the calcium supplement in this study, in which 25 mmol (1000 mg) Ca was provided once daily, 5 d/wk, was similar to that observed in several studies in which the supplement was consumed in small doses taken several times a day (Table 6). Concerns have been expressed that the efficacy of calcium supplements may be reduced if consumed in doses > 400–500 mg/d (7). Although there is convincing evidence that this may be the case in short-term studies (18–20), this suggests that it may not apply to long-term supplementation. Given the potential compliance problems associated with split-dose regimens, more research is required to establish whether the efficacy of calcium supplementation over a prolonged period is influenced by dose schedule.

This longitudinal study of Gambian women demonstrated strikingly that renal conservation of calcium occurs during lactation, as has been suggested from previous studies (1). In the placebo group, urinary calcium output at 3 mo (0.62 mmol/d, or 25 mg/d) was approximately one-half that at 1.5 wk and that of a separate group of NPNL Gambian women. Urinary calcium output increased slowly in later lactation and at 78 wk was similar to that at 1.5 wk. It is not possible from this study to determine whether the renal calcium conservation noted in the months after delivery was associated specifically with lactation or was a consequence of pregnancy.

There was no evidence that the extent of renal conservation was modified by current calcium intake. Because the difference in urinary calcium output between the supplement and placebo
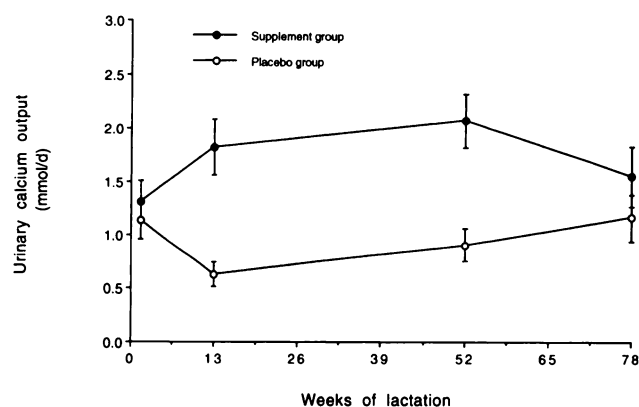
TABLE 6
Effect of changes in calcium intake on urinary calcium excretion[1]

Calcium salt	Brand[2]	Number	Subjects[3]	Dose	Schedule[4]	Duration	Δ Urine calcium[5]	Urine response	Reference
				mmol/d			mmol/d		
Carbonate	Calcichew	30	F, L	17.9	S, B	12 mo	1.19[a]	6.6%	This study
Carbonate	Caltrate	13	F, PM	25.0	S, N	1 d	1.45[b]	5.8%	13
Carbonate	Calsup	13	F, PM	30.0	S, N	1 d	1.42[b]	4.7%	13
Carbonate	—	28	F, PM	12.5	S, N	24 mo	0.58[a,c]	4.6%	24
Carbonate	—	6	M+F	39.0	D, N	4 d	2.35	6.0%	15
Carbonate	—	5	M+F	77.0	D, N	4 d	4.65	6.0%	15
Citrate	Citracal	14	F	25.0	D, N	6 mo	1.60	6.4%	17
Citrate malate	Citrus Hill + Ca	25	F, PM	12.5	S, N	24 mo	0.73[a,c]	5.8%	24
Lactogluconate	Sandocal	36	F, PM	25.0	S, N	24 mo	0.80[d]	3.2%	25
Lactogluconate	Sandocal	13	F, PM	25.0	S, N	1 d	1.37[b]	5.5%	13
Lactogluconate-carbonate	—	61	F, PM	25.0	D, N	6–24 mo	1.00[e]	4.0%	26
Diet + carbonate	—	57	M+F	—	—	—	—	6.0%[f]	15, 16
Diet, low/high	—	21	F	—	—	—	—	2.9%[f]	27
Diet, habitual	—	104	F	—	—	—	—	2.2%[g]	28

[1] Results relate to measurements of urinary calcium excretion in collections lasting ≥ 24 h. Multiply by 40 to convert mmol Ca/d to mg/d.

[2] Calcichew, Shire Pharmaceuticals Ltd, Hampshire, UK; Caltrate, Lederle, Sydney, Australia; Calsup, Riker, Sidney, Australia; Citracal, Mission Pharmacal, San Antonio, TX; Citrus Hill + Ca, Procter and Gamble, Cincinnati; Sandocal, Sandoz, Basel, Switzerland.

[3] M, male; F, female; L, lactation; PM, postmenopause.

[4] S, single daily dose; D, daily dose divided into two to four portions; B, consumed between meals; N, timing of dose in relation to meals not discussed.

[5] a, Difference between supplement and placebo group; b, in group of high calcium absorbers; c, calculated from data expressed relative to creatinine, assuming a creatinine output of 9 mmol/d; d, difference between two exercise groups with and without calcium; e, estimated from graph; f, slope of regression line of change in urinary calcium against change in calcium intake; g, slope of regression line of calcium output against habitual calcium intake.

groups at 3 and 12 mo was equivalent to that predicted from studies in nonlactating individuals (*see* above, Table 6), it seems likely that, had the women in the supplement group received the supplement at 1.5 and 78 wk, their urinary calcium output at those time points would have been increased by a similar amount. If this were the case, the lactational changes in urinary calcium output experienced by the supplemented women would parallel those seen in the placebo group. Interestingly, the urinary calcium outputs of British mothers at 3 mo of lactation were more than double those of the supplemented Gambian women despite their similar total calcium intakes. This may reflect differences in diet composition or the intake of certain nutrients, or in adaptive or racial differences in calcium metabolism and renal physiology.

In addition to urinary calcium output, significant changes in forearm BMC were observed within individuals during lactation, irrespective of calcium intake, decreasing at 3 mo of lactation and returning to baseline by 12 mo. The effect was small (1%), but, if reflected throughout the skeleton, would represent a release of ≈125–250 mmol (5–10 g) Ca in 3 mo, equivalent to ≈1.25–2.5 mmol/d (50–100 mg/d).

The calcium concentration in Gambian breast milk was low compared with British values, as was noted previously (3). In addition, breast-milk calcium concentrations decreased within individuals as lactation progressed, averaging 5.2 mmol/L (21 mg/dL) at 13 wk and 3.7 mmol/L (15 mg/dL) at 78 wk. Because Gambian women produce ≈750 mL and 500 mL breast milk/d at these time points (21), the women in this study will have required ≈3–4 mmol/d (120–160 mg/d) for breast-milk secretion. The observed changes in BMC, coupled with renal calcium conservation of ≈0.5–1 mmol/d (20–40 mg/d), could, therefore, have made a sizeable contribution to calcium supply for breast-milk production. It is possible that calcium

economy may have been improved further by increases in calcium absorption efficiency and decreases in dermal and gastrointestinal calcium losses. In addition, although previous studies of bone mobilization in lactating women have been inconsistent (1), recent investigations suggest that certain skeletal sites, particularly the spine, may respond more than others (22, 23). If this were the case, it would suggest that, in the Gambian women, a high proportion, if not all, the calcium required for breast-milk production was provided by such physiological adjustments.

Low breast-milk calcium concentrations have been observed in some nonindustrialized regions of the world, but not others (1, 3). This study demonstrated that the low concentrations typical of Gambian breast milk do not appear to be a function of maternal calcium intake during lactation. There is evidence to suggest that calcium intake during pregnancy may be important (29), as may the concentration of other milk components such as citrate and casein. This question will be explored in future studies.

In summary, this study demonstrated that, in lactating women accustomed to a low-calcium diet, temporary adjustments in renal and bone physiology occur that may furnish a substantial proportion of the calcium needed for breast-milk production, and that an increase in current calcium intake has no effect on breast-milk calcium concentration or forearm BMC. These results have implications for the determination of recommended calcium intakes for lactating women, but further research is required to establish whether similar results are obtained in women on a higher plane of calcium nutrition. ◼

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