The effect of long-term calcium supplementation on indices of iron, zinc and magnesium status in lactating Gambian women

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The effect of long-term supplementation with $CaCO_3$ on indices of Fe, Zn and Mg status was investigated in a randomized, double-blind intervention study of sixty lactating Gambian women. The supplement contained 1000 mg Ca and was consumed between meals 5 d/week, for 1 year starting 1·5 weeks postpartum. Compliance was 100%. Plasma ferritin concentration, plasma Zn concentration and urinary Mg output were measured before, during and after supplementation at 1·5, 13, 52 and 78 weeks postpartum. No significant differences in mineral status were observed at any time between women in the supplement and placebo groups. Analysis of the longitudinal data series showed that plasma ferritin and Mg excretion were characteristic of the indivdiual ($P < 0.001$). Within individuals, ferritin concentration was higher at 1·5 weeks postpartum than later in lactation ($P = 0.002$). Plasma Zn concentration was lower at 1·5 weeks postpartum than at other times ($P < 0.001$), an effect which disappeared after albumin correction. Low plasma concentrations of ferritin and Zn indicated that the Gambian women were at high risk of Fe and Zn deficiency. Measurements of α_1-antichymotrypsin suggested that the results were not confounded by acute-phase responses. The results of the present study indicate that 1000 mg Ca as $CaCO_3$ given between meals does not deleteriously affect plasma ferritin and Zn concentrations or urinary Mg excretion in women who are at risk of Fe and Zn deficiency.

Calcium: Mineral interactions: Lactation

High intakes of Ca have been associated with a decreased risk of osteoporosis, hypertension, pre-eclampsia, hypercholesterolaemia, diabetes mellitus and certain cancers (Levenson & Bockman, 1994). The use of Ca supplements is an increasingly popular method of increasing dietary intake, and in the USA, for example, total annual sales exceed $200 million per annum (Gossel, 1991; Levenson & Bockman, 1994). There is evidence suggesting that Ca absorption may be greater and more consistent if supplements are consumed with food rather than in the fasting state (Heaney, 1991; Levenson & Bockman, 1994), and frequently advice is given to ingest Ca supplements at meal-times (Levenson & Bockman, 1994). However, it has long been recognized that high Ca intakes may adversely affect the absorption and status of other minerals, especially Fe (Hallberg *et al.* 1992) and Zn (Argiratos & Samman, 1994). This is a potential concern, especially for individuals who are at risk of becoming deficient in these minerals. From the evidence available the effect appears to be related to mineral interactions in the lumen of the gastrointestinal tract or to competition between minerals for absorptive pathways (Fairweather-Tait, 1995). One

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possible strategy to minimize mineral interactions while avoiding potential problems associated with the fasting state would be to consume Ca supplements between meals. However, the effects of Ca supplements consumed in this way on the status of other minerals has been little studied (Whiting, 1995).

An opportunity arose during a detailed investigation of Ca requirements of lactating women (Prentice *et al.* 1995) to examine whether the long-term, regular ingestion of a $CaCO_3$ supplement affects Fe, Zn and Mg status, when consumed between the mid-day and evening meals. Sixty Gambian mothers took part in a randomized, double-blind, placebo-controlled, supplementation study during prolonged lactation. The supplement, 1000 mg Ca as $CaCO_3$, or placebo was consumed daily on 5 d/week for 12 months. The aim of the work reported here was to study the effect of the Ca supplement on plasma Zn and ferritin concentrations and on urinary Mg excretion during and after the supplementation period.

SUBJECTS AND METHODS
Subjects and study design

Sixty lactating mothers were enrolled in the study between March 1990 and March 1991. The mothers were resident in Keneba and Manduar, two villages in a rural region of The Gambia, West Africa, and were 16–41 years old (mean 28 (SD 8) years), parity 1–13 (mean 5 (SD 3)). The women breast-fed their infants, fully and on demand, for more than 18 months, as is traditional in this society, with the provision of complementary foods from about 3–4 months. The study was approved by the MRC Gambia Ethics Committee and informed consent was obtained from all subjects.

All the women were taking part in a detailed longitudinal investigation of Ca requirements of lactating women habituated to a low-Ca diet (Prentice *et al.* 1995). The aim of the larger study was to determine, by means of a randomized, placebo-controlled trial, the effect of Ca supplementation on bone mineralization, lactational performance and efficiency of Ca absorption in lactating women accustomed to a low dietary Ca intake (7·1 mmol/d (283 mg/d); Fairweather-Tait *et al.* 1995; Prentice *et al.* 1995). Baseline measurements were made on 9 (SD 1) d postpartum. On the following day the women were randomly assigned, double-blind, to either a Ca-supplement or placebo-control group. Since baseline measurements were made on approximately four subjects each month, a permuted block of four was used for the group allocation to minimize the possibility of seasonal bias.

The supplements, consisting either of two chewable $CaCO_3$ tablets (Calcichew; Shire Pharmaceuticals Ltd, Andover, Hants; 12·5 mmol (500 mg) elemental Ca per tablet) or two tablets of dextrose of similar taste and texture (Dextro-energy; CPC Ltd, Esher, Surrey), were provided 5 d/week for the subsequent 12 months. The tablets were delivered to the subjects each day by fieldworkers who supervised their consumption. The supplements were consumed in the early evening between 17.00 and 19.00 hours. They were taken at least 2 h after lunch (generally eaten at 14.00–15.00 hours) and at least 1 h before dinner (eaten about 20·00 hours). During the fast month of Ramadan, tablets were consumed later in the evening once the subjects had broken fast but before their main meal. Tablets missed because of illness or absence from the village were taken at weekends. Over the total supplementation period, compliance was 100 % (i.e. every subject consumed all the tablets that had been allocated to her, supervised by the fieldworker) and the Ca intake of the supplemented group was raised by an average of 17·9 mmol/d (714 mg/d).

There were no differences between women in the supplement (S) and placebo (P) groups in terms of age (years; S 28 (SD 7), P 28 (SD 8)), parity (S 5 (SD 3), P 6 (SD 3)), weight at baseline (kg; S 53·9 (SD 5·7), P 55·9 (SD 8·9)), height (m; S 1·59 (SD 0·05), P 1·60 (SD 0·06)),

or dietary Ca intake at 13 weeks (mmol/d; S 6·9 (SD 2·9), P 7·2 (SD 3·2)). Further details of the subjects and the supplementation study are described elsewhere (Prentice *et al.* 1995).

Plasma and urine for the assessment of Zn, Fe and Mg status were obtained at baseline and at 13, 52 and 78 weeks postpartum (d; mean 92 (SD 6), 366 (SD 5) and 547 (SD 5)). Blood from the antecubital vein was collected after an overnight fast into cooled lithium heparin tubes, centrifuged within 45 min, the plasma separated and stored at $-20°$. Urine was collected over a 24 h period. All urine containers and apparatus were acid-washed to minimize mineral contamination. A fieldworker visited the subject at the start and end of the collection period, and at regular intervals during the day to collect filled urine bottles and to deliver them to the laboratory refrigerator. The urine fractions for each subject were pooled, mixed, and the total volume recorded. The portion for Mg analysis was acidified with concentrated HCl (Spectrosol; BDH, Poole, Dorset) to a final concentration of 0·12 mol/l (10 ml/l) and stored at $-20°$. The plasma and urine samples were transported to the Cambridge laboratory on dry ice for analysis.

Laboratory assays

Plasma ferritin was measured by ELISA using a modification of a published method (Anderson & Kelly, 1981). The modifications were: diluting rabbit antiferritin antibody 1:400 with coating buffer, diluting the antibody–horseradish peroxidase (EC 1.11.1.7) conjugate 1:15000 with bovine serum albumin, diluting the ferritin standards with bovine serum albumin. Antisera and human liver ferritin standards were obtained from Dako Ltd, High Wycombe, Bucks. Bovine serum albumin, fraction V, was purchased from Sigma Chemicals Ltd, Poole, Dorset. The lower limit of the reference range for plasma ferritin was taken as 12 μg/l (Fairweather-Tait, 1993).

Plasma Zn was measured colorimetrically using a commercial kit method (Wako Chemicals GmbH, Nauss, Germany) with a Cobas-Bio centrifugal analyser (Roche Products Ltd, Welwyn Garden City, Herts.). The method is based on the formation of a coloured complex between Zn and '5-Br-PAPS' (2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)-phenol) and salicylaldoxime, after deproteinization with trichloracetic acid. This assay was evaluated against flame atomic absorption spectroscopy and the two methods were found to be in close agreement.

Urinary Mg concentrations were measured with the Cobas-Bio centrifugal analyser using an enzymic method (Tsang *et al.* 1988). Reagents were purchased from Sigma Chemicals Ltd and the $Mg(NO_3)_2$ standard from BDH Ltd, Poole, Dorset. The assay was linear below 0·82 mmol/l (20 mg/l). Samples and standards were diluted as necessary with HCl (0·12 mol/l; 10 ml/l; Spectrosol, BDH Ltd).

The plasma proteins albumin and α_1-antichymotrypsin were measured by immuno-nephelometric methods with the Cobas-Bio centrifugal analyser using specific antisera, calibrators and reagents purchased from Dako Ltd. The α_1-antichymotrypsin assays were conducted on the samples collected at 13 weeks only. The upper limit of the reference range for α_1-antichymotrypsin concentration, an acute-phase marker, was taken as 0·64 g/l (Calvin *et al.* 1988).

Creatinine concentration was measured in plasma and urine to determine creatinine clearance rate. The samples were assayed using a commercial centrifugal analyser kit based on the kinetic buffered Jaffé reaction without deproteinization (Roche Products Ltd). A creatinine clearance rate of < 60 ml/min per 1·73 m^2 was taken as an indication of incomplete urine collection (Prentice *et al.* 1995). Rates below this threshold were obtained for two, four and twelve subjects at 13, 52 and 78 weeks respectively, and the daily Mg outputs for these subjects were omitted from final data analysis.

Reference quality-control materials were included with all batches of analyses to monitor

accuracy and precision. The materials used were: plasma Zn, Certified Reference Human Serum (J. Versieck, University of Ghent, Belgium); plasma ferritin, Lyphocheck Immunoassay Control (Bio-Rad Laboratories Ltd, Hemel Hempstead, Herts.); urinary Mg and creatinine, Lyphocheck Normal Urine Control (Bio-Rad Laboratories Ltd); plasma creatinine, Roche Control Serum N (Roche Products Ltd); plasma albumin, Seronorm Protein (Nycomed Pharma A/S, Oslo, Norway); plasma α_1-antichymotrypsin, Human Serum Protein Control (Dako Ltd). Observed values for all reference materials were within the quoted ranges provided by the producers. Measurements of between-batch reproducibility were 3 % (at 12 μmol/l), 5 % (at 30 μg/l) and 2 % (at 4 mmol/l) for the Zn, ferritin and Mg assays respectively. Staff conducting the laboratory analyses in Cambridge were blind to the treatment-group assignments.

Comparative data

Comparative data were obtained at 13 weeks postpartum (88 (SD 8) d) from fifteen lactating British women living in Norwich, Norfolk (Prentice *et al.* 1995). These women were 22–41 years old, parity 1–4 (age 29 (SD 5) years, parity 2 (SD 1)). Eight mothers were exclusively breast-feeding, seven were also providing their infants with small quantities of formula milk, other drinks or solid foods. The mothers were heavier and taller than the Gambian subjects (weight 64·6 (SD 11·0) kg; height 1·64 (SD 0·06) m). Identical techniques were used for the collection of plasma and urine as in the Gambian study, and the samples were analysed alongside the Gambian samples.

Statistical analysis

Statistical analyses were performed by ANOVA (repeat measures, nested form), analysis of covariance and linear regression using Linear Model software on DataDesk 4.1 (Data Description Inc., 1993). The Data Desk approach is suitable for use with unbalanced datasets; missing datapoints are excluded from analyses. Scheffé *post hoc* tests were used to test the significance of differences between groups of data. Analyses of plasma ferritin results were conducted after logarithmic transformation to correct a marked positive skew in the distibution. Possible seasonal effects were explored by dividing each year into three seasons: wet (July–October), harvest (November–February) and hot (March–June).

Exploration of possible effects of the supplement were examined in two ways. First, by constructing a model using the measure of interest at either 13, 52 or 78 weeks as dependent variable, with its value at baseline, season and supplementation group as independent variables. Second, by constructing a model with the measure of interest as dependent variable and with subject, supplementation group, lactation stage and season as independent variables. The first approach investigates differences between women in the S and P groups at each lactation stage, after adjusting for initial value and season. The second approach examines the possible effect of the supplement on time trends within subjects. Group differences observed with both methods, examined using the Scheffé *post hoc* test, are presented as means and 95 % CI.

RESULTS

No significant differences were observed at any time in the indices used to assess Zn, Fe and Mg status between women receiving the Ca supplement and those receiving the placebo (Tables 1 and 2). The observed mean differences were small, generally < 10 % of the total value (Table 2). The two statistical approaches used to examine the effect of the supplement gave similar results (Table 2). In addition, there was no significant difference in plasma albumin between the groups (Table 1).

Concentrations of the acute-phase marker, α_1-antichymotrypsin, measured at the 13 weeks time point, were similar for women in the S and P groups and no subject had a

Table 1. *Effect of a calcium supplement on plasma and urinary indices of mineral status in lactating Gambian women**

(Mean values and standard deviations, with ranges shown in parentheses)

Stage of lactation (weeks) …	Baseline S		Baseline P		13 S		13 P		52 S		52 P		78 S		78 P	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma: n	30		30		30		30		30		30		30		30	
Zn† (μmol/l)	8.8	3.0	9.3	2.3	10.5	2.0	10.1	1.9	10.5	2.6	10.5	2.2	11.2	1.8	10.8	2.1
	(3.7–14.2)		(4.5–13.8)		(5.2–13.8)		(5.6–14.8)		(4.8–14.7)		(4.8–13.8)		(6.8–16.7)		(6.3–14.2)	
Albumin (g/l)	27.2	5.0	28.4	4.0	37.0	2.8	37.2	3.1	37.3	4.9	35.7	4.3	38.7	5.7	37.4	5.4
	(14.4–36.1)		(18.8–34.0)		(29.5–42.5)		(29.5–45.2)		(28.3–50.9)		(26.9–44.1)		(28.5–49.7)		(22.5–48.9)	
Ferritin‡ $\log_{10}$ (μg/l)	1.31	0.42	1.20	0.37	1.23	0.36	1.13	0.44	1.17	0.36	1.10	0.35	1.15	0.39	1.06	0.32
	(0.50–2.26)		(0.48–1.98)		(0.46–2.24)		(0.41–2.03)		(0.49–2.20)		(0.51–1.87)		(0.55–2.32)		(0.55–1.71)	
Urine: n	28		29		29		26		26		26		23		18	
Mg† output (mmol/d)	31.3	17.7	28.5	9.6	31.6	12.0	32.8	17.6	31.9	14.2	29.4	20.3	36.6	16.5	29.5	18.4
	(1.3–83.0)		(11.6–52.1)		(13.9–61.8)		(9.0–73.7)		(9.8–60.0)		(9.1–108.5)		(10.3–76.5)		(10.4–84.6)	
Mg†: creatinine (mmol/mmol)§	0.46	0.25	0.42	0.14	0.45	0.15	0.44	0.22	0.46	0.21	0.41	0.25	0.52	0.30	0.53	0.48
	(0.04–0.95)		(0.17–0.81)		(0.22–0.74)		(0.15–1.05)		(0.15–0.94)		(0.12–1.37)		(0.18–1.44)		(0.15–2.28)	

S, supplement group; P, placebo group.

* Supplementation was for 52 weeks following baseline measurements at 9 d postpartum. For details of subjects and procedures, see pp. 822–824. There were no significant differences between women in the S and P groups at any time.

† To convert Zn to μg/l and Mg to mg/l multiply by 65.4 and 24.3 respectively.

‡ Geometric mean ferritin concentrations (μg/l) at baseline: S 20.5, P 15.9; 13 weeks S 17.1, P 13.3; 52 weeks S 14.8, P 12.5; 78 weeks S 14.0, P 11.5.

§ Subjects with creatinine clearance $\geq$ 60 ml/min per 1.73 m².

Table 2. *Differences between calcium-supplemented (S) and placebo (P) groups of lactating Gambian women in plasma and urinary indices of mineral status after statistical adjustment**

(Values are mean differences S−P and 95% CI for sixty subjects)

	Plasma Zn (μmol/l)	Plasma ferritin ($\log_{10}$; μg/l)	Plasma ferritin† ($\log_{10}$; μg/l)	Urinary Mg (mmol/d)
Model 1				
13 weeks	+0·6	+0·04	−0·01	−2·4
	(−0·5–+1·7)	(−0·13–+0·21)	(−0·18–+0·16)	(−10·4–+5·6)
52 weeks	+0·4	+0·02	−0·05	+1·2
	(−0·7–+1·5)	(−0·13–+0·17)	(−0·22–+0·12)	(−8·6–+11·0)
78 weeks	+0·4	+0·04	+0·02	+8·7
	(−0·6–+1·4)	(−0·12–+0·20)	(−0·15–+0·19)	(−1·1–+18·5)
Model 2				
All time points	+0·1	+0·09	+0·02	+2·8
	(−0·5–+0·7)	(−0·08–+0·26)	(−0·13–+0·17)	(−2·4–+8·0)

Model 1; effect of S group on the measured variable at each time point after adjusting for baseline value and season using analysis of covariance; model 2; effect of S group on the measured variable after adjusting for subject, season and lactation stage using repeat measures analysis of variance (nested form).

* Supplementation was for 52 weeks following baseline measurements at 9 d postpartum. For details of subjects and procedures, see pp. 822–824. No difference was statistically significantly different from 0 ($P > 0.05$).

† Subjects with plasma ferritin concentration $\geqslant$ 12 μg/l at all time points, n 30.

concentration above the reference range. The mean values in the two groups were: supplemented 0·35 (SD 0·06, range 0·26–0·56) g/l; placebo 0·33 (SD 0·006, range 0·22–0·52) g/l.

A substantial proportion of Gambian mothers had a plasma ferritin concentration below 12 μg/l, indicating depleted Fe stores. The percentage of subjects with low ferritin concentrations was 33 at baseline, 43 at 13 weeks, 44 at 52 weeks and 50 at 78 weeks. No effect of the Ca supplement on plasma ferritin was observed if the data were restricted to mothers with plasma ferritin concentrations above 12 μg/l (Table 2).

In the longitudinal dataset, plasma ferritin concentration and urinary Mg excretion were characteristic of the individual across the four time points, but this was not observed for plasma Zn and albumin concentrations (ferritin $P < 0.001$, Mg output, $P < 0.001$, Mg:creatinine $P < 0.001$, Zn $P = 0.08$, albumin $P = 0.21$). Within individuals there were highly significant effects of stage of lactation, with plasma Zn and albumin concentrations being 15% lower at day 9 than later in lactation ($P < 0.001$), and plasma ferritin levels being 10% higher ($P = 0.002$). For example, the mean differences within individuals between measurements at 52 weeks compared with those at baseline were: ferritin −0·13 (95% CI −0·21–−0·05) μg/l ($\log_{10}$), Zn +1·5 (95% CI +0·7–+2·3) μmol/l, albumin +8·7 (95% CI +7·2–+10·2) g/l. Plasma Zn concentrations were highly correlated with plasma albumin levels (Zn (μmol/l) = 3·0 + 0·21 × albumin (g/l); $P < 0.001$), and the effect of lactation stage on Zn disappeared once the data were adjusted for albumin using analysis of covariance ($P = 0.54$; mean difference at 52 weeks compared with baseline −0·1 (95% CI −1·0–+0·8) μmol/l). No correlations were observed between α_1-antichymotrypsin levels and either plasma Zn, with or without albumin correction, or plasma ferritin concentrations measured at week 13. Urinary Mg excretion was not influenced by lactation stage ($P = 0.26$).

Within individuals, after adjustment for lactation stage, plasma Zn concentration varied between the seasons, with hot-season values higher than at other times of year ($P < 0.001$;

Table 3. *Effect of season on indices of zinc and magnesium status in lactating Gambian women**

(Values are mean differences and 95% CI between seasons, within individuals after adjusting for lactation stage using repeat measures analysis of variance. Further adjustment for albumin was by analysis of covariance. Differences between seasons were obtained using Scheffé *post-hoc* tests. Data are for sixty women measured four times during lactation)

Seasonal difference...	Hot − Harvest	Hot − Wet	Wet − Harvest	Statistical significance overall: P
Plasma Zn‡ (μmol/l)	+1·5 (+0·7−+2·3) $P < 0·001$	+1·1 (+0·3−+1·9) $P = 0·013$	+0·4 (−0·4−+1·2) NS	< 0·001
Plasma albumin (g/l)	+2·6 (+1·1−+4·1) $P = 0·003$	+4·5 (+3·1−+5·9) $P < 0·001$	−1·9 (−3·5−−0·3) NS	< 0·001
Plasma Zn‡ adjusted for albumin (μmol/l)	+1·1 (+0·4−+1·8) $P = 0·008$	+0·2 (−0·6−+1·0) NS	+0·9 (+0·2−+1·6) $P = 0·047$	0·004
Urinary Mg‡ output (mmol/d)†	+6·4 (+0·8−+12·0) NS	+11·5 (+5·7−+17·3) $P < 0·001$	−5·1 (−11·1−+0·9) NS	< 0·001
Urinary Mg‡: creatinine† (mmol/mmol)	+0·15 (+0·07−+0·23) $P = 0·003$	+0·17 (+0·09−+0·25) $P < 0·001$	−0·02 (−0·12−+0·08) NS	< 0·001

Hot, March–June; Wet, July–October; Harvest, November–February.

* For details of subjects and procedures, see pp. 822–824.

† Urine values are for subjects with creatinine clearance $\geqslant$ 60 ml/min per 1·73 m². Missing data points are as shown in Table 1.

‡ To convert Zn to μg/l and Mg to mg/l multiply by 65·4 and 24·3 respectively.

Table 3). This was associated in part with seasonal variations in albumin concentration (Table 3). However, after adjusting for albumin using analysis of covariance, a significant effect of season on plasma Zn concentration remained ($P = 0·004$), with harvest-season values lower than at other times. Urinary Mg excretion was higher in the hot season than during the rest of the year (Table 3). No seasonal variation in plasma ferritin or α_1-antichymotrypsin concentrations was evident. No differences were observed between S and P groups either in absolute values or in the pattern of change across time for any of the indices of mineral status, after statistical adjustment for the possible confounding effects of lactation stage and season (Table 2).

Table 4 compares the indices of mineral status between lactating Gambian and British mothers at 13 weeks postpartum. The Gambian women had a significantly lower plasma Zn concentration than the British mothers. This was partly accounted for by lower albumin concentration, but a substantial difference in Zn concentration remained after adjusting for albumin using analysis of covariance (mean difference Gambian − British −2·7 (95% CI −3·9−−1·5) μmol/l, $P < 0·001$). The concentration of α_1-antichymotrypsin was lower in the British women (Table 4) but adjusting for α_1-antichymotrypsin did not affect the between-country difference in Zn concentration. There were no significant differences in plasma ferritin or urinary Mg excretion between women in the two countries. As with the Gambian subjects, a substantial percentage (36) of the British women had plasma ferritin concentrations < 12 μg/l at 13 weeks postpartum.

One Gambian mother had a consistently raised ferritin concentration which exceeded

Table 4. *Comparison between indices of mineral status in lactating Gambian and British mothers at 13 weeks postpartum**

(Mean values and standard deviations, with ranges shown in parentheses for sixty Gambian and fifteen British women)

	Gambian		British		Statistical significance of difference: P
	Mean	SD	Mean	SD	
Plasma Zn† (μmol/l)	10·3	2·0	13·4	2·3	< 0·001
	(5·2–14·8)		(10·0–17·6)		
Plasma albumin (g/l)	37·1	2·9	40·6	7·1	0·004
	(29·5–45·2)		(28·0–49·6)		
Plasma ferritin ($\log_{10}$; μg/l)‡	1·18	0·40	1·17	0·33	NS (0·92)
	(0·41–2·24)		(0·60–1·73)		
Plasma α_1-antichymotrypsin (g/l)	0·34	0·06	0·25	0·03	< 0·001
	(0·22–0·56)		(0·20–0·31)		
Urinary Mg† output (mmol/d)§	31·8	14·9	41·1	11·7	NS (0·06)
	(7·1–73·7)		(17·7–58·7)		
Urinary Mg†: creatinine (mmol/mmol)§	0·45	0·18	0·37	0·13	NS (0·13)
	(0·15–0·56)		(0·15–1·05)		

* For details of subjects and procedures, see pp. 822–824.
† To convert Zn to μg/l and Mg to mg/l multiply by 65·4 and 24·3 respectively.
‡ Geometric mean ferritin concentrations (μg/l): Gambian 15·1, British 14·7.
§ Subjects with creatinine clearance $\geqslant$ 60 ml/min per 1·73 m² (n 58 Gambian women, n 15 British women).

150 μg/l at all time points but was not associated with an elevated α_1-antichymotrypsin concentration. Analysing the data without this subject did not alter the interpretation of results in any way.

DISCUSSION

Concerns have been raised about the possible detrimental effects of increased Ca intake on the status of other minerals, particularly Fe (NIH Consensus Statement, 1994; Whiting, 1995). An inhibitory effect of Ca on the absorption of Fe has been demonstrated in a number of studies (Dawson-Hughes *et al.* 1986; Cook *et al.* 1991; Hallberg *et al.* 1992; Whiting, 1995), an effect which appears to be particularly striking when Ca supplements are consumed with meals (Cook *et al.* 1991; Whiting, 1995). Effects of Ca on Zn and Mg absorption have also been identified (Wacker & Vallee, 1964; Ferguson *et al.* 1989; Argiratos & Samman, 1994). In spite of these detailed experimental studies, there have been few investigations of the long-term implications of raised Ca intake on the status of other minerals. Short-term studies, ranging from several days to 3 months, have not identified any effect of Ca supplementation on Fe status or Zn retention (Spencer *et al.* 1984; Dawson-Hughes *et al.* 1986; Sokoll & Dawson-Hughes, 1992). The study of lactating Gambian women presented here has demonstrated that regular ingestion of a $CaCO_3$ supplement for 12 months, consumed between meals, does not result in measurable changes in plasma Zn concentration, plasma ferritin concentration and urinary Mg excretion.

The supplementation study was designed principally to investigate whether lactating women on low-Ca diets would benefit from an increase in Ca intake in terms of breast-milk Ca concentration and bone mineral content (Prentice *et al.* 1995). The sample size (n 30 per group), therefore, was determined by power calculations based on the main outcome variables and was not selected to test whether the Ca supplementation alters the status of other minerals. However, using calculations based on the data for lactating British women

at 13 weeks (Table 4), the study had the statistical power ($P < 0.05$ and 80 % power) to detect differences between the S and P groups of 12 % for plasma Zn and 20 % for plasma ferritin and urinary Mg output. Such differences, had they been observed, would have indicated a modest effect of the supplement, similar to the detected changes with season and lactation stage. Smaller effects may have been missed with this sample size, but would have been of minor physiological importance.

The results of the present study are limited by the indices of mineral status chosen for measurement. It is possible, for example, that other factors, homeostatic or environmental, may have influenced the concentrations of plasma ferritin and Zn and the excretion of Mg, obscuring the effects of the Ca supplement on other body pools of these minerals. In addition, plasma ferritin is a sensitive measure of residual Fe stores, but is of relatively little value in investigating change in depleted subjects (plasma ferritin concentration < 12 μg/l; Fairweather-Tait, 1993). Measurements of circulating transferrin receptor might have been useful in this respect but were not available in the present study. However, analysis of the ferritin data in those Gambian women with plasma ferritin concentrations $\geqslant 12$ μg/l did not suggest any detrimental effect of the supplement on Fe stores (Table 2).

The Ca intake of the Gambian women in the present study, in common with other women in this part of Africa, was low compared with international recommendations (Prentice, 1994). The $CaCO_3$ supplement, 25 mmol/d (1000 mg/d) taken 5 d/week, tripled the Ca intake of these women for over 12 months (Prentice et al. 1995). The Ca supplement was well received, compliance was 100 %, and the observed increase in urinary Ca output, reflecting a higher quantity of Ca absorbed, was in line with the response predicted from other studies (Prentice et al. 1995). In addition, the efficiency of Ca absorption of the Gambian women was high compared with that of the British mothers, as measured by a double-stable-isotope technique (Fairweather-Tait et al. 1995).

The women recruited into the supplementation study were considered to be at high risk of Fe deficiency, because of the high prevalence of Fe-deficiency anaemia in this population. This proved to be the case, with more than 40 % of the women at 13 weeks postpartum having a plasma ferritin concentration below 12 μg/l, the lower limit of the reference range (Fairweather-Tait, 1993). This percentage increased to 50 % by 78 weeks postpartum.

Although the measurement of plasma Zn concentration has limitations as an index of Zn status (Aggett & Favier, 1993), the biochemical results suggested that the Gambian subjects were also at risk of Zn deficiency, since their plasma Zn concentration at 13 weeks postpartum was substantially lower than that of the comparative group of lactating British women (by 3.1 and 2.7 μmol/l before and after albumin correction respectively). The mean plasma Zn concentration of the British women, 13.4 μmol/l, was similar to published data for breast-feeding mothers in other well-nourished communities (Moser & Reynolds, 1983; Karra et al. 1988; Krebs et al. 1995). The lower plasma Zn concentration at 9 d postpartum compared with later in lactation has been observed in other groups of women who have recently given birth (Moser & Reynolds, 1983; Karra et al. 1988; Krebs et al. 1995). In the Gambian women this was accounted for entirely by reduced plasma albumin concentration in the period immediately postpartum.

Assessments of Zn and Fe status using plasma Zn and ferritin concentration are confounded by infection, since both are influenced by the acute-phase response. Plasma Zn concentration tends to fall, and plasma ferritin concentration to rise, during episodes of infection or inflammation. However, the low concentrations of α_1-antichymotrypsin, a sensitive acute-phase marker (Calvin et al. 1988), in the plasma samples collected at 13 weeks, and the consistency of plasma ferritin concentrations within individuals over the 18-month period, suggest that the results were not affected by episodes of infection. The

seasonal variations observed in plasma Zn concentration may have been associated with changes in exposure to infective stress, activity levels and/or diet but, since they occurred in the group as a whole, they are unlikely to have obscured differences between women receiving the Ca supplement and those receiving the placebo.

International recommendations for optimal Ca nutrition vary, but many include advice to increase Ca intake during puberty, pregnancy and lactation (Prentice, 1991, 1994). Anxieties about osteoporosis have led to calls for large increases in the recommendations for Ca intake particularly for adolescents and postmenopausal women (NIH Consensus Statement, 1994). For many, especially for individuals who do not regularly consume milk and dairy products, such high Ca intakes are not readily achievable by dietary means. Ca supplements and Ca-fortified foods are becoming increasingly popular as a means to increase Ca intake. Experts are divided about the optimal timing of Ca supplements, with some arguing that they should be consumed with meals in order to improve efficiency of absorption (Levenson & Bockman, 1994), while others advocate consumption away from meals to minimize possible adverse interactions with other minerals and trace elements (NIH Consensus Statement, 1994). The present randomized supplementation study of lactating mothers in The Gambia has demonstrated that long-term ingestion of $CaCO_3$ between meals by healthy women at high risk of Fe deficiency, and possibly also of Zn deficiency, does not influence Fe, Zn and Mg status as assessed by ferritin and Zn concentrations in plasma and by urinary Mg excretion.

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