

# Bone changes after 3 mo of lactation: influence of calcium intake, breast-milk output, and vitamin D-receptor genotype<sup>1-3</sup>

M Ann Laskey, Ann Prentice, Lisa A Hanratty, Landing MA Jarjou, Bakary Dibba, Sian R Beavan, and Tim J Cole

See corresponding editorial on page 591.

**ABSTRACT** Factors influencing the change in bone mineral after 3 mo of lactation were investigated in 47 breast-feeding mothers, 11 formula-feeding mothers, and 22 nonpregnant, nonlactating control subjects. At 6–8 wk postpartum, the breast-feeding group had a mean ( $\pm$ SD) calcium intake of  $34.8 \pm 13.2$  mmol/d and breast-milk volume, calcium concentration, and calcium output of  $0.865 \pm 0.230$  L/d,  $7.41 \pm 1.25$  mmol/L, and  $6.41 \pm 2.00$  mmol/d, respectively. There was no relation between calcium intake and any breast-milk variable. Dual-energy X-ray absorptiometry of the whole body, spine, hip, and forearm was performed at 0.5 and 3 mo. There were significant decreases in bone mineral content at the spine (3.96%; 95% CI: 4.86%, 3.06%), femoral neck (2.39%; 95% CI: 3.61%, 1.17%), total hip (1.51%; 95% CI: 2.45%, 0.60%), and whole body (0.86%; 95% CI: 1.29%, 0.43%) in breast-feeding mothers but not in formula-feeding mothers or nonpregnant, nonlactating women. These changes were not related to calcium intake, breast-milk calcium concentration, vitamin D-receptor genotype, postpartum weight change, or use of the progesterone-only contraceptive pill. After adjustment for bone area, breast-milk volume and height were identified as significant predictors at the spine, such that greater decreases were associated with taller mothers ( $P = 0.007$ ) and those with greater breast-milk volume ( $P = 0.001$ ). This finding suggests that the marked bone mineral changes observed in breast-feeding mothers represented a physiologic response to lactation that was independent of dietary calcium supply. *Am J Clin Nutr* 1998;67:685–92.

**KEY WORDS** Lactation, bone mineral content, dietary calcium, breast-milk calcium, breast-milk volume, vitamin D-receptor genotype, women

## INTRODUCTION

A breast-feeding mother secretes  $\approx 5.0$ – $7.5$  mmol (200–300 mg) Ca into breast milk every day, and this can be as high as 10 mmol/d (400 mg/d) in some individuals (1). This represents a substantial proportion of the calcium intake of many lactating women (2). Several recent studies have shown that breast-feeding is associated with decreases in bone mineral and that effects are more evident at skeletal sites rich in trabecular bone (3–9). The calcium released from the skeleton may provide some of the extra calcium required for breast-milk production. Little is known about the factors that affect the skeletal response to lac-

tation in individual women. However, it is plausible that the magnitude of the effect may be related to the calcium requirement of the mother and may be influenced by her calcium intake and her breast-milk calcium secretion. Several recent studies found no relation between the calcium intake of the mother and bone mineral changes during lactation (3, 10, 11).

Although there is some evidence that the decreases in bone mineral may be reversed toward the end of lactation and after weaning (3–7, 9), there is concern that lactational bone loss may increase the risk of osteoporosis during lactation or after menopause, particularly in women with low bone status (12–17). It is important, therefore, to characterize women who have large decreases in bone mineral during lactation and to determine whether any factor can affect these changes so that appropriate recommendations can be made.

The aim of the study was to investigate the relations between bone mineral changes after 3 mo of lactation and the mother's calcium intake, breast-milk calcium concentration, breast-milk volume, postpartum weight change, contraceptive pill use, and other factors. Data on the mother's vitamin D-receptor (VDR) genotype were also included because there is evidence that nutrient-gene interactions affect calcium and bone metabolism (18, 19). The results are from the initial phase of a longitudinal study designed to follow women throughout lactation and into the postweaning period.

## SUBJECTS AND METHODS

### Volunteers

Healthy white women were eligible for the study if they lived in the Cambridge area, were aged 20–40 y, and had recently given birth to a healthy full-term infant. Mothers were recruited postnatally in the local maternity hospital with the intention of following them for at least the first 12 mo postpartum. Data are

<sup>1</sup> From the MRC Dunn Nutrition Centre, Cambridge, United Kingdom.

<sup>2</sup> Supported in part by the Sainsbury Charitable Fund.

<sup>3</sup> Address reprint requests to MA Laskey, MRC Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 1XJ, United Kingdom. E-mail: ann.laskey@mrc-dunn.cam.ac.uk.

Received February 2, 1997.

Accepted for publication November 7, 1997.

presented here for 47 mothers who breast-fed for  $\geq 3$  mo (BF) and for 11 formula-feeding (FF) mothers. Two FF mothers had breast-fed for 2–3 d, the others had never breast-fed. In addition, 22 nonpregnant, nonlactating (NPNL) women of similar age were recruited through advertisement or by word of mouth. Potential volunteers were not included if they had a history of bone disease or were taking medications known to affect bone. Smoking and use of an oral contraceptive were not criteria for exclusion. Approval for the study was obtained from the Ethical Committee of the MRC Dunn Nutrition Unit and informed, written consent was obtained from each subject.

Women visited the Dunn Clinical Nutrition Centre for baseline bone mineral measurements and other investigations as soon as possible after discharge from the hospital:  $17 \pm 5$  d ( $\bar{x} \pm$  SD) postpartum (range: 10–42 d) and for repeat measurements at 3 mo postpartum ( $90 \pm 4$  d; range: 83–103 d). The mean time interval between the baseline and the 3-mo measurements was  $73 \pm 7$  d (range: 49–89 d). In addition, breast-milk output and food diary and other measurements were completed in the subject's home at 6–8 wk postpartum ( $53 \pm 9$  d; range: 38–72 d).

No advice was given to the volunteers about their own diet or that of their infants. Mothers breast-fed for as long as they wished and introduced solid foods when they felt it was appropriate. Detailed information about infant growth and infant-feeding practice (daily number of breast-feedings, number of formula feedings, and number of meals including weaning foods) was collected at each visit. At 6–8 wk no BF mothers had introduced their infants to any weaning foods, but five were giving their infants one or more bottles of formula milk per day. By 3 mo, 10 mothers had introduced bottles of formula milk to their infants. Two of these mothers and 10 other mothers had just started to give their infants small amounts of solid foods. For this analysis, 42 mothers were defined as fully breast-feeding at 6–8 wk and 37 mothers at 3 mo in accordance with the definition of Lobbok and Krasovec (20).

### Bone mineral measurements

Bone mineral content (BMC; in g) and bone area (BA; in  $\text{cm}^2$ ) of the whole body, lumbar spine (L1–L4), left hip, and nondominant forearm were measured by dual-energy X-ray absorptiometry (DXA) (QDR-1000/W; Hologic Inc, Waltham, MA). The performance mode was used for spine and hip measurements (software V4.47P) and software V5.61 was used for the whole body (enhanced analysis) and forearm. The 3-mo scan was analyzed with reference to the individual's baseline image by using the DXA compare facility. Quality assurance and long-term instrument stability were assessed by using the Hologic spine phantom, which was scanned at the beginning of each measurement day. Over the 3 y of the study, the CV of phantom measurements for both BMC and BA was  $<0.4\%$  and there was no indication of any significant drift over time. Estimates of in vivo precision of BMC and BA-adjusted BMC (21), determined from two sets of scans from NPNL women at an interval of  $\approx 3$  mo were as follows: spine, 1.3% and 0.9%; femoral neck, 3.0% and 2.3%; trochanter, 6.3% and 2.2%; total hip, 4.2% and 1.2%; whole body, 0.7% and 0.5%; radial wrist, 1.6% and 1.4%; and radial shaft, 1.3% and 1.2%.

### Breast milk

Breast-milk volume was determined 6–8 wk after parturition and before the infants started to receive any solid food. Mea-

surements were made by administering deuterium oxide to the mother, which quantifies breast-milk volume over 14 d (22, 23). Twenty-nine mothers successfully completed these measurements. In addition, 12 elected to use the test-weighing method over a period of  $\geq 24$  h (23) and 6 did not complete breast-milk volume measurements.

A sample of breast milk ( $<2$  mL) was collected from each breast at 6–8 wk by manual expression into calcium-free, disposable plastic tubes and stored at  $-20^\circ\text{C}$  for compositional analysis. The samples were collected at the mother's convenience because breast-milk calcium concentration is not influenced by time of day or stage of feed (1). Whole milk samples were analyzed for calcium by using a semiautomated spectrophotometric method validated against atomic absorption (24). Quality assurance was achieved by including a commercial reference material (bovine milk powder, SRM 1549; National Institute of Standards and Technology, Gaithersburg, MD) plus an aliquot of a pooled sample of breast milk with each batch of samples (24).

### Calcium intake

Calcium intake from the diet plus that from supplements and medications was estimated at baseline and at 6–8 wk and 3 mo postpartum by a food-frequency questionnaire (FFQ), which probed for recent consumption of calcium-rich food sources in the UK diet, particularly dairy products, calcium-enriched flour, and vegetables (Calquest; Department of Food and Nutritional Sciences, King's College, London) (25). In addition, a prospective 7-d food diary that used photographs to assist in the selection of portion sizes was completed at 6–8 wk (26). Coding and computation of nutrient intakes from the diary was undertaken by using the DIDO and MWIN4 software programs, based on British food-table data (27).

The validity of the food diaries was assessed by expressing energy intake (EI) as a multiple of estimated basal metabolic rate ( $\text{BMR}_{\text{est}}$ ). EI was determined from the food diary and  $\text{BMR}_{\text{est}}$  was calculated by using Schofield equations (28). Allowance was made for the energy associated with any weight change during the study period and for the energy required for breast-milk production (29–31). A ratio of EI to  $\text{BMR}_{\text{est}}$  ( $\text{EI}:\text{BMR}_{\text{est}}$ ) of 1.2 was selected as the lower limit because most of the mothers reported very sedentary lifestyles (30). If the  $\text{EI}:\text{BMR}_{\text{est}}$  was  $<1.2$ , it was concluded that the subject had underreported EI and quite possibly calcium intake.

### Vitamin D-receptor genotype

Blood was collected for VDR genotyping from 37 BF mothers. DNA was extracted from whole blood (Nucleon Kit; Scot-Lab, Coatbridge, Scotland) and stored at  $-20^\circ\text{C}$  until needed. Polymerase chain reaction (PCR) was performed by using primers described previously (32). Genotype was determined by digestion of the PCR product using *BsmI* endonuclease (Promega, Southampton, United Kingdom). The number and position of bands was determined in 2% agarose gels, visualized by ultraviolet light after ethidium bromide staining (32).

### Statistical analyses

Statistical analysis was performed by using analysis of variance (ANOVA), analysis of covariance, and multiple regression analysis (linear model software, DATADESK 4.1; Data Description Inc, Ithaca, NY). Repeated-measures ANOVA was used to

investigate the effect of time on calcium intake within individuals. The significance of differences between data pairs was calculated by using the post hoc Scheffé test. Seasonal effects were investigated after subjects were grouped according to season at entry into the study: January–March, April–June, July–September, and October–December. Contraceptive pill use was coded as a binary variable (1/0).

BMC was adjusted for BA by using regression analysis rather than by calculating areal bone mineral density (BMD; in g/cm<sup>2</sup>), to avoid incomplete BA correction (21). All continuous variables, except age, were converted to natural logarithms to facilitate exploration of power relations between continuous variables and examination of proportional effects of discrete factors (21, 33). The regression coefficient for a discrete variable when the dependent variable is in natural logarithms, once multiplied by 100, corresponds closely to the percentage effect as defined by (difference/mean) × 100 (33). All percentage differences quoted in the text were derived in this manner.

Multiple regression analysis with backwards elimination of nonsignificant variables was performed to identify determinants of the change in bone mineral after 3 mo of lactation. Initial BMC was included as an independent variable in all models to adjust for regression toward the mean, except when examining the influence of mean BMC. The possible effect of variation between women in the timing of the baseline and 3-mo measurements and in the time interval between them was examined by including the difference and mean days postpartum in the regression analyses. Neither variable was significant in any regression model and are not discussed further.

## RESULTS

### Subject characteristics

The baseline characteristics of the subjects in the three groups are shown in **Table 1**. NPWL women were younger on average than BF mothers but the age ranges of the two groups were similar. The bone status of the three groups was similar and close to the manufacturer's reference appropriate for their age (*z* score near 0; Table 1). There were no significant differences in weight and height between any of the groups. One FF mother had a baseline weight of 140 kg. When her data were omitted, the mean weight for the FF mothers was close to that of the BF mothers.

By 3 mo, BF mothers had lost weight ( $\bar{x} \pm SD$ :  $1.7 \pm 3.4\%$  of baseline weight,  $P = 0.001$ ) but it varied between individuals

( $-7.6\%$  to  $6.1\%$ ). FF mothers lost  $2.5 \pm 3.4\%$  of baseline weight ( $P = 0.035$ ), which was not significantly different from the weight loss of BF mothers ( $P = 0.25$ ). There was no significant weight change in NPWL women. At 3 mo, only 1 BF mother had resumed menstruation and 10 were using a progesterone-only oral contraceptive that they had been taking for  $50 \pm 19$  d. The VDR genotype frequency of BF mothers was as follows: *BB*, 19%; *Bb*, 43%; and *bb*, 38%. This distribution was in Hardy-Weinberg equilibrium and was comparable with that in other reports in populations of North European ancestry (34).

### Calcium intake

Calcium intake, as estimated by an FFQ, decreased between baseline and 3 mo and differed between groups, with the BF mothers tending to have a higher calcium intake than the FF mothers and NPWL women (**Table 2**). Calcium intake at 6–8 wk was assessed by both an FFQ and a food diary. When all records were included, the food diary tended to give a lower result than the FFQ for all groups (Table 2). There was a significant correlation between the two estimates for BF mothers ( $r = 0.65$ ,  $P = 0.0001$ ) and NPWL women ( $r = 0.52$ ,  $P = 0.015$ ), but not for FF mothers ( $r = 0.34$ ,  $P = 0.38$ ). For some individuals there were large differences between the two estimates (FFQ – diary in BF mothers:  $3.7 \pm 10.3$  mmol/d). Calculated EI:BMR<sub>est</sub> was  $\geq 1.2$  for only 26 of 45 BF mothers, 2 of 9 FF mothers, and 15 of 20 NPWL women, suggesting underreporting in many of the diaries. Restricting the food-diary data to those subjects with an EI:BMR<sub>est</sub>  $\geq 1.2$  increased the calculated calcium intake for all groups (Table 2) but did not improve the correlation between the FFQ and food-diary data (BF mothers:  $r = 0.59$ ,  $P = 0.002$ ; NPWL women:  $r = 0.46$ ,  $P = 0.10$ ) or the prediction of one estimate from the other (FFQ – diary in BF mothers:  $4.1 \pm 11.7$  mmol/d). Because there were no criteria to indicate that one method was superior to another for the estimation of calcium intake, further analyses of results related to calcium intake at 6–8 wk were investigated three ways: FFQ data, food-diary data, and food-diary data restricted to those subjects with an EI:BMR<sub>est</sub>  $\geq 1.2$ .

### Breast-milk calcium output

Breast-milk volume, breast-milk calcium concentration, and calculated daily breast-milk calcium output at 6–8 wk are given in **Table 3**. There was considerable variation in all three variables between individuals (Table 3). Breast-milk volume was influenced by the size of the infant. At 6–8 wk, breast-milk volume was positively correlated with infant weight ( $P < 0.0001$ ) and length ( $P = 0.006$ ), although, when considered together, only

**TABLE 1**  
Subject characteristics at baseline

	Breast-feeders ( <i>n</i> = 47)	Formula-feeders ( <i>n</i> = 11)	NPWL women ( <i>n</i> = 22)
Age (y)	32.0 ± 4.0 <sup>1</sup>	29.5 ± 3.4	27.7 ± 6.9 <sup>2</sup>
Height (m)	1.65 ± 0.06	1.67 ± 0.06	1.65 ± 0.06
Weight (kg)	67.6 ± 10.5	75.8 ± 24.5 <sup>3</sup>	65.0 ± 15.2
Parity	1–2	1–3	0–4
Spine <i>z</i> score	−0.21 ± 1.13	0.24 ± 1.54	0.09 ± 0.86
Femoral neck <i>z</i> score	−0.58 ± 1.25	−0.20 ± 1.27	−0.41 ± 0.92

<sup>1</sup>  $\bar{x} \pm SD$ . NPWL, nonpregnant, nonlactating.

<sup>2</sup> Significantly different from breast-feeders,  $P = 0.0008$ .

<sup>3</sup> One subject had a baseline weight of 140 kg. Omission of her data gave a mean weight similar to that of breast-feeders.

**TABLE 2**  
Calcium intake of volunteers<sup>1</sup>

	Breast-feeders	Formula-feeders	NPNL women
	<i>mmol/d</i>		
Food-frequency questionnaire <sup>2</sup>			
Baseline	37.0 ± 13.2 [47] (14.0–77.3)	27.7 ± 5.3 [11] (18.6–39.1)	25.3 ± 10.1 [22] (7.8–48.2)
6–8 wk	34.8 ± 13.2 [45] (14.0–69.2)	29.5 ± 10.7 [11] (14.5–47.4)	24.0 ± 9.9 [20] (7.8–48.6)
3 mo	32.4 ± 10.0 [47] (11.2–57.2)	25.9 ± 13.3 [11] (13.2–61.3)	25.4 ± 9.8 [22] (10.1–52.4)
7-d Food diary			
All subjects	31.4 ± 10.4 [45] (15.9–57.0)	16.7 ± 3.9 [9] <sup>3</sup> (10.0–21.6)	22.6 ± 4.9 [20] <sup>3</sup> (11.4–29.0)
Subjects with ratio ≥ 1.2 <sup>4</sup>	36.2 ± 9.8 [26] (17.5–57.0)	19.8 ± 0.5 [2] (19.5–20.2)	24.1 ± 3.7 [15] <sup>3</sup> (17.2–29.0)

<sup>1</sup>  $\bar{x}$  ± SD; range in parentheses; *n* in brackets. NPNL, nonpregnant, nonlactating.

<sup>2</sup> Significant differences by two-factor, repeated-measures ANOVA in calcium intake across time ( $P = 0.036$ ) and between groups ( $P = 0.0009$ ). Significance of differences obtained by Scheffé post hoc tests were 1) effect of time: 3 mo vs baseline,  $P = 0.038$ ; 6–8 wk vs baseline, NS; 3 mo vs 6–8 wk, NS; 2) effect of group: breast-feeders vs NPNL,  $P = 0.003$ ; breast-feeders vs formula-feeders,  $P = 0.06$ ; formula-feeders vs NPNL,  $P = 0.77$ . There was no evidence of an interaction between groups and time effects ( $P = 0.26$ ).

<sup>3</sup> Significantly different from breast-feeders,  $P < 0.001$  (ANOVA with Scheffé post hoc tests).

<sup>4</sup> Subjects with a ratio of energy intake to estimated basal metabolic rate ≥ 1.2.

infant weight was an independent predictor ( $P = 0.001$ ). There was no relation between breast-milk volume and maternal height or weight ( $P = 0.92$  and  $0.10$ , respectively). Breast-milk volume and calcium concentration were not significantly correlated ( $P = 0.81$ ) and neither was influenced by the mother's age ( $P = 0.86$  and  $0.32$ , respectively) or VDR genotype ( $P = 0.96$  and  $0.44$ , respectively). The results were similar when only fully breast-feeding mothers were considered.

No significant correlations ( $P > 0.6$ ) were observed between a mother's calcium intake and her breast-milk volume, calcium concentration, and total calcium output. Similar results were obtained with calcium intake estimated by FFQ or food diary ( $\pm$  EI:BMR<sub>est</sub> ≥ 1.2) and with subjects confined to those who were fully breast-feeding.

### Bone mineral changes after 3 mo lactation

At 3 mo, BMC decreased significantly in BF mothers at the lumbar spine, femoral neck, total hip, and whole body but not at the trochanter, radial shaft, or wrist (**Table 4**). The marked decreases in BMC at the spine, hip, and whole body were still apparent after adjustment for BA (**Figure 1**). Because no change was observed at the trochanter, the effect at the total hip reflected the decrease in bone mineral at the femoral neck. This differential effect of lactation was also observed in the regional

analysis of the whole-body scan, which showed significant decreases in both BMC and BA-adjusted BMC at the thoracic spine, lumbar spine, and pelvis, but not in the arms or legs (**Figure 2**). There was no significant effect of time interval between scans on these changes in BMC and the results were similar with and without the inclusion of mothers with the smallest and largest time intervals. There was no overall difference in BA between baseline and 3 mo at any region ( $P > 0.05$ ), although there was variation within individuals. No significant changes in BMC or BA-adjusted BMC were observed at any bone site in either FF mothers or NPNL women (**Table 4**). Because volunteers entered the study at different times during the calendar year, the possibility that the bone results were affected by seasonal variation was investigated. No significant effect of season on within-individual variations in BMC or BA-adjusted BMC were discernible by ANOVA among BF mothers, FF mothers, or NPNL women ( $P > 0.1$ ).

### Heterogeneity of bone response to lactation

There was wide variation in the percentage change ( $\Delta\%$ ) in bone mineral at the spine, femoral neck, and whole body after 3 mo of lactation (spine:  $\Delta$ BMC =  $-9.4\%$  to  $2.4\%$ ,  $\Delta$ BA-adjusted  $\Delta$ BMC =  $-8.3\%$  to  $2.2\%$ ; femoral neck:  $\Delta$ BMC =  $-12.2\%$  to  $6.4\%$ ,  $\Delta$ BA-adjusted  $\Delta$ BMC =  $-10.9\%$  to  $3.3\%$ ; and whole body:  $\Delta$ BMC =  $-4.8\%$  to  $1.4\%$ ,  $\Delta$ BA-adjusted  $\Delta$ BMC =  $-3.7\%$  to  $1.6\%$ ; **Figure 3**). The extent to which these variations reflected differences between individuals in response to lactation as opposed to other factors such as measurement error and short-term biological change was assessed by reference to NPNL data (**Figure 3**). At the spine, the residual variance ( $s^2$ ) of  $\Delta$ BA-adjusted  $\Delta$ BMC was nearly four times greater for BF mothers ( $s^2 = 6.00$ ) than for NPNL women ( $s^2 = 1.66$ ), indicating that 72% of the variation in the BF group could be ascribed to differences in the response to lactation and 28% to other factors. Similar calculations showed that 49% of the variance in  $\Delta$ BA-adjusted  $\Delta$ BMC at the whole body and practically all of that at the femoral neck could be accounted for by factors unrelated to lactation.

**TABLE 3**  
Breast-milk volume, calcium concentration, and calcium output at 6–8 wk<sup>1</sup>

	All mothers	Fully breast-feeding mothers
Volume (L/d)	0.865 ± 0.230 [41] (0.340–1.500)	0.890 ± 0.220 [37] (0.607–1.500)
Calcium concentration (mmol/L)	7.41 ± 1.25 [45] (5.19–10.46)	7.42 ± 1.33 [40] (5.20–10.46)
Calcium output (mmol/d)	6.41 ± 2.00 [41] (2.56–11.25)	6.61 ± 1.96 [37] (3.25–11.25)

<sup>1</sup>  $\bar{x}$  ± SD; range in parentheses. *n* in brackets. There were no significant differences,  $P > 0.05$ .

TABLE 4

Change in bone mineral content after 3 mo<sup>1</sup>

	Breast-feeders (n = 47)	Formula-feeders (n = 11)	NPNL women (n = 22)
		%	
Lumbar spine	-3.96 (-4.86, -3.06) <sup>2</sup>	-1.03 (-2.66, 1.60)	0.44 (-0.30, 1.18)
Femoral neck	-2.39 (-3.61, -1.17) <sup>2</sup>	-0.35 (-3.49, 2.79)	0.47 (-1.31, 2.25)
Trochanter	-0.58 (-2.42, 1.38)	0.28 (-3.01, 3.57)	2.20 (-1.52, 5.92)
Total hip	-1.51 (-2.45, -0.60) <sup>3</sup>	0.35 (-1.02, 1.72)	0.68 (-1.38, 2.74)
Whole body	-0.86 (-1.29, -0.43) <sup>2</sup>	0.27 (-0.83, 1.37)	0.01 (-0.40, 0.42)
Radial wrist	-0.33 (-1.02, 0.36)	1.13 (-0.36, 2.62)	-0.39 (-1.37, 0.59)
Radial shaft	-0.14 (-0.69, 0.41)	0.61 (-0.90, 2.12)	0.49 (-0.25, 1.23)

<sup>1</sup> Data are mean percentage differences from baseline; 95% CIs in parentheses. NPNL, nonpregnant, nonlactating.<sup>2,3</sup> Significantly different from zero: <sup>2</sup>  $P < 0.001$ , <sup>3</sup>  $P < 0.005$ .

### Determinants of change in bone mineral status at 3 mo

Independent determinants of  $\Delta$ BMC at the spine, femoral neck, and whole body were explored in multiple regression models on logged data (see Methods). Adjustment for BA was achieved by including  $\Delta$ BA while regression toward the mean was minimized by incorporating initial BMC.  $\Delta$ BMC was positively correlated with  $\Delta$ BA at all three sites ( $P \leq 0.0001$ ); regression toward the mean was significant only at the femoral neck ( $P = 0.003$ ).

At the spine,  $\Delta$ BMC was negatively correlated with maternal height and breast-milk volume, indicating that the percentage decrease in bone mineral was greater for taller women and for those producing the larger volume of breast milk (Table 5). The  $s^2$  for the model in Table 5 was 4.28, indicating that these two variables accounted for 29% of the variance in  $\Delta$ BA-adjusted  $\Delta$ BMC ( $s^2 = 6.00$ , see above). There was no significant effect of breast-milk calcium concentration on  $\Delta$ BMC ( $P = 0.67$ ), and the use of breast-milk calcium output in place of breast-milk volume in the regression model did not increase the significance of the relation ( $P = 0.005$  compared with 0.001). This suggests that the change in BMC at the spine was associated with the amount of breast milk produced and not specifically with the amount of calcium secreted.

No other significant, independent determinants of  $\Delta$ BMC were observed at the spine. The variables considered were maternal calcium intake at 6–8 wk as measured by FFQ ( $P = 0.83$ ) or by food diary (all subjects:  $P = 0.43$ ; subjects with an EI:BMR<sub>est</sub>  $\geq 1.2$ :  $P = 0.50$ ), change in weight ( $P = 0.18$ ), mean weight ( $P = 0.28$ ), mean BMC ( $P = 0.75$ ), mean BA ( $P = 0.51$ ), age ( $P = 0.09$ ), parity ( $P = 0.54$ ), contraceptive pill use ( $P = 0.91$ ), infant length ( $P = 0.11$ ), infant weight ( $P = 0.97$ ), infant length gain ( $P = 0.11$ ), and infant weight gain ( $P = 0.76$ ).

The only independent predictors of  $\Delta$ BMC at the femoral neck, after adjustment for  $\Delta$ BA and initial BMC, were maternal weight and mean BA (ln weight: coefficient  $\pm$  SE =  $0.117 \pm 0.042$ ,  $P = 0.008$ ; ln mean BA: coefficient  $\pm$  SE =  $0.098 \pm 0.045$ ,  $P = 0.033$ ), indicating that lighter women and those with a smaller BA had a greater percentage decrease in BMC. No significant determinant of  $\Delta$ BMC other than  $\Delta$ BA was identified for the whole body.

There were no significant effects of VDR genotype on change in bone mineral at any skeletal site, before or after adjustment for change in BA and other determinants ( $P > 0.05$ ). For example, at the lumbar spine, the mean ( $\pm$ SE) difference between the genotypes in percentage change in  $\Delta$ BA-adjusted  $\Delta$ BMC was as follows:  $BB - Bb = 0.22 \pm 1.58\%$ ,  $BB - bb = 1.27 \pm 1.32\%$ , and  $Bb - bb = 1.05$

$\pm 1.08\%$  ( $P = 0.45$ ). In addition, there was no influence of VDR genotype on BA-adjusted BMC or BA at any site ( $P > 0.05$ ) or on maternal weight and height ( $P = 0.26$  and  $0.14$ , respectively).

### DISCUSSION

This study confirmed previous observations that lactation is associated with a decrease in bone mineral and that this effect is particularly pronounced at the spine and femoral neck (3–6, 9, 10, 35). The inclusion of subregional analyses of total hip and whole-body scans further emphasized the fact that there are variations in the response to lactation between different parts of the skeleton. This study also showed that the observed decrease in bone mineral was associated with lactation per se and was not a consequence of the mother having recently been pregnant because no bone change was observed postpartum in FF mothers. In addition, the results indicate that these changes were not related to seasonal variation in bone mineral status, in agreement with one study in the United States (3) but in contrast with another in Spain (36).

The bone mineral change that was observed over 3 mo of breast-feeding was much greater and more rapid than the average

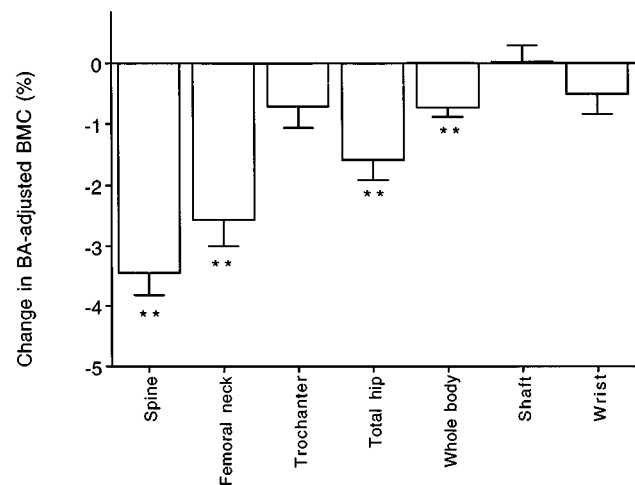
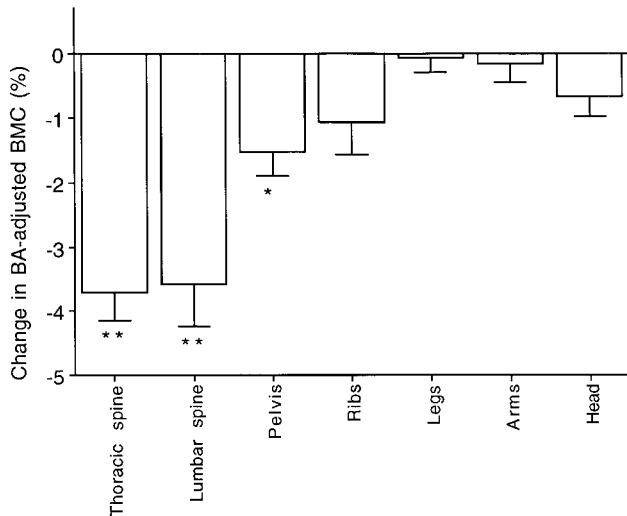


FIGURE 1. Mean ( $\pm$  SEM) percentage change in bone area (BA)-adjusted bone mineral content (BMC) from 0.5 to 3 mo lactation at the spine, femoral neck, trochanter, total hip, whole body, radial shaft, and radial wrist in 47 mothers.  $**P < 0.005$ .



**FIGURE 2.** Mean ( $\pm$  SEM) percentage change in bone area (BA)-adjusted bone mineral content (BMC) from 0.5 to 3 mo lactation in sub-regions of the whole body: thoracic spine, lumbar spine, pelvis, ribs, legs, arms, and head in 47 breast-feeding mothers. \* $P < 0.01$ , \*\* $P < 0.005$ .

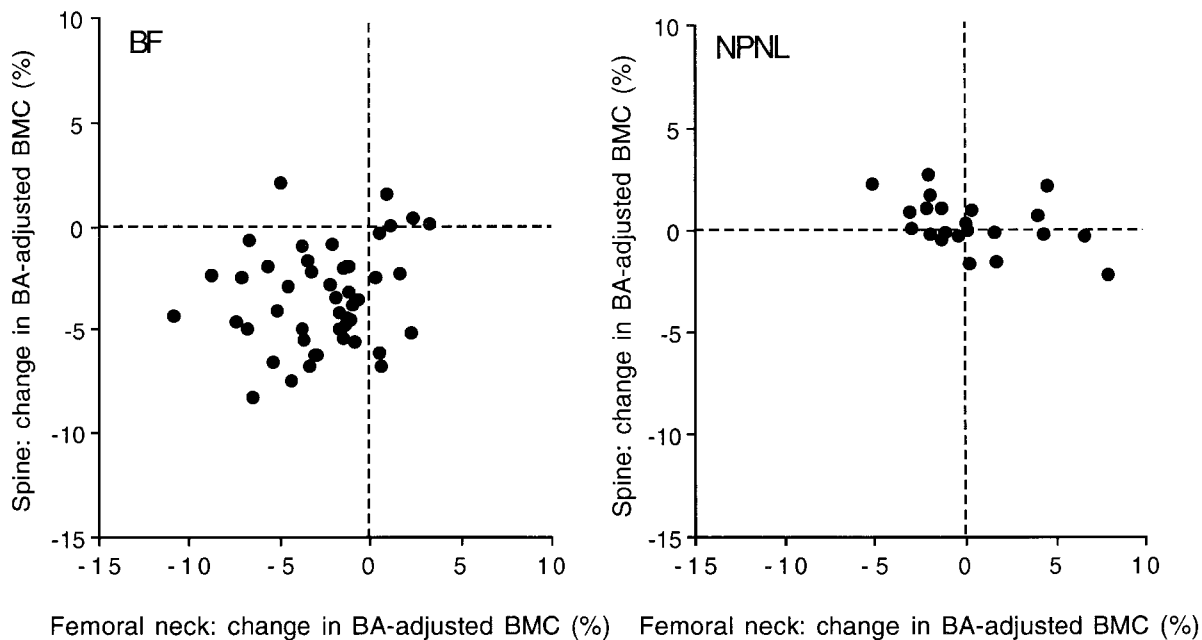
bone loss experienced by postmenopausal women, but like at menopause, the magnitude of the change varied considerably from one individual to another (37). This study examined the extent to which this variation reflected differences in the calcium stress on the individual mother in terms of her dietary calcium supply and her breast-milk calcium output.

The calcium intake of the BF mothers was moderate to high, similar to that reported in a previous study in Cambridge (38), but varied over a greater than fourfold range. Calcium intake was assessed by two well-established techniques: an FFQ and a 7-d food diary. Agreement between the methods was only modest,

especially at the individual level. In principle, the food diary provides detailed information over a defined period whereas the FFQ records consumption of specified calcium-rich foods in the recent past. Although most of the subjects who volunteered for this intensive study were highly educated and well motivated, calculations indicated that a proportion of them had underreported their EI in the food diary. Removing their data, however, did not improve the agreement between the FFQ and food-diary estimates of an individual's calcium intake, although the mean values for the group more closely resembled those obtained by the FFQ. In consequence, analyses examining the influence of diet on bone mineral change were conducted separately with all three estimates of calcium intake because there was no means of determining which was most correct.

No relations were observed between maternal calcium intake, estimated by any of the three methods, and the magnitude of the bone mineral change at any site. This supports the growing body of evidence that indicates that the skeletal response to lactation is independent of maternal calcium intake (3, 6, 39). For example, decreases in bone mineral similar to those in the mothers from Cambridge were observed in lactating women with calcium intakes ranging from low ( $< 20$  mmol/d) to high ( $> 40$  mmol/d) (5, 10, 35). In addition, four recent studies showed that calcium supplementation does not alter the pattern of bone mineral change during lactation, even in women accustomed to a very low calcium intake (5, 10, 35, 40). Only one study has reported an association between dietary calcium and the effect of lactation on bone: adolescent mothers who increased their calcium intake after receiving dietary advice to do so had no change in forearm bone mineral whereas a similar group who received no advice showed a marked decrease in bone mineral (41).

There were wide variations in the breast-milk volume, breast-milk calcium concentration, and breast-milk calcium output of mothers in this study at 6–8 wk of lactation, even in those who were exclusively breast-feeding. No relations were observed



**FIGURE 3.** Percentage change in bone area (BA)-adjusted bone mineral content (BMC) from 0.5 to 3 mo lactation at the spine and femoral neck in 47 breast-feeding (BF) and 22 nonpregnant, nonlactating (NPNL) women.

**TABLE 5**

Multiple regression model examining the influence of breast-milk volume and maternal height on percentage change in bone mineral content (BMC) at the spine in 41 mothers breast-feeding for > 3 mo<sup>1</sup>

Variable	Coefficient	SEM	t ratio	P
Constant	176	50	3.53	0.001
ln Initial BMC	-0.001	0.021	-0.06	0.95
$\Delta$ ln BA	1.021	0.220	4.65	> 0.0001
ln Height	-0.293	0.102	-2.86	0.007
ln Volume	-0.044	0.013	-3.48	0.001

<sup>1</sup> Results are for measurements made at baseline and 3 mo. Dependent variable =  $\Delta$  ln BMC,  $R^2$  adjusted = 58%, df = 36,  $s^2$  = 4.28.


between any of these breast-milk variables and the mother's calcium intake despite the fact that breast-milk calcium output represented  $\approx 22\%$  of maternal calcium intake and was >40% for some women. This finding agrees with previous studies that found no association between maternal calcium intake and breast-milk calcium concentration (39) and with a recent study in Gambian women that showed that calcium supplementation during lactation had no effect on breast-milk calcium concentration (40).

Breast-milk volume was shown to be a major determinant of bone mineral change in the lumbar spine such that mothers producing the larger volume of breast-milk at 6–8 wk had the greater decrease in bone mineral over 3 mo of lactation. This result suggests that the skeleton may react to signals produced directly or indirectly in response to suckling. There was no evidence that the decrease in bone mineral was associated specifically with the secretion of breast-milk calcium. The mobilization of bone, however, could make a significant contribution to the calcium required for breast-milk production. In this study, whole-body BMC averaged 2200 g and decreased on average by 0.86% in 72 d. This corresponded to a release of  $\approx 2.5$  mmol Ca/d from the skeleton, which is equivalent to 39% of the breast-milk calcium output (6.4 mmol/d) at 6–8 wk.

The only other independent determinants of lactational bone change identified in this study were related to the mother's bone and body size:  $\Delta$ BA at all sites (positive), height at the lumbar spine (negative), weight, and mean BA at the femoral neck (positive). The decrease in bone mineral was not affected by use of the progesterone-only contraceptive pill. This finding contrasts with that from a study in Scotland that reported that users of the progesterone-only pill had smaller decreases in spine bone density during lactation (4). No effect of postpartum weight change was observed, unlike studies investigating the effects of weight loss in nonlactating, obese adults (42). In addition, the VDR genotype of the mother did not influence the skeletal response at any site, in contrast with studies of bone loss in postmenopausal and elderly women (18, 19).

The mechanism by which bone loss occurs during lactation is unclear but may be related to the sudden decrease in estrogen concentration after parturition to a concentration similar to that found after menopause and to changes in other hormones, such as prolactin. Elevated bone-formation markers (plasma osteocalcin and bone-specific alkaline phosphatase) and bone-resorption markers (hydroxyproline, deoxypyridinoline, and *N*-telopeptide) have been observed in lactating women, indicating that the decrease in bone mineral is related to an increase in bone resorption rather than to depressed bone formation (5, 7, 43, 44). There is evidence that parathyroid hormone-related peptide, produced by the mammary gland and possibly under the influence of pro-

lactin, may play a key role in regulating maternal calcium and bone metabolism during lactation, especially during the early weeks (45–47). The involvement of such a factor, produced in response to suckling, is consistent with our finding that breast-milk volume is a major determinant of lactational bone loss at the spine.

In summary, this study showed that marked changes occur in the skeleton of mothers during 3 mo of lactation and that the response differs between individuals and between different regions of the skeleton. The magnitude of the effect is related to the amount of breast milk consumed by the infant and by several factors relating to maternal size. No lifestyle determinants were identified. In particular, the bone changes were independent of the calcium intake of the mother in the range 14–70 mmol/d. Further work to elucidate the mechanisms that determine an individual's skeletal response to lactation may provide a valuable insight into the regulation of bone metabolism and may help to explain why some individuals are more vulnerable to postmenopausal osteoporosis than others. 

We thank the many members of the MRC Dunn Nutrition Unit who facilitated this research; RG Whitehead, AJ Crisp, and EM Widdowson for their unfailing enthusiasm and encouragement; and all the volunteers and their families for their commitment and interest, to whom we are indebted.

## REFERENCES

1. Laskey MA, Prentice A, Shaw J, et al. Breast-milk calcium concentrations during prolonged lactation in British and rural Gambian mothers. *Acta Paediatr Scand* 1990;79:507–12.
2. Prentice A. Maternal calcium requirements during pregnancy and lactation. *Am J Clin Nutr* 1994;59(suppl):477S–83S.
3. Sowers MF, Corton G, Shapiro B, et al. Changes in bone density with lactation. *JAMA* 1993;269:3130–5.
4. Caird LE, Reid-Thomas V, Hannan WJ, Gow S, Glasier AF. Oral progestogen-only contraception may protect against loss of bone mass in breast-feeding women. *Clin Endocrinol* 1994;41:739–45.
5. Cross NA, Hillman LS, Allen SH, Krause GF. Changes in bone mineral density and markers of bone remodeling during lactation and postweaning in women consuming high amounts of calcium. *J Bone Miner Res* 1995;10:1312–20.
6. Kalkwarf HJ, Specker BL. Bone mineral loss during lactation and recovery after weaning. *Obstet Gynecol* 1995;86:26–32.
7. Affinito P, Tommaselli GA, Di Carlo C, Guida F, Nappi C. Changes in bone mineral density and calcium metabolism in breastfeeding women: a one year follow-up study. *J Clin Endocrinol Metab* 1996;81:2314–8.
8. Laskey MA, Hanratty LA, Prentice A. Regional bone changes during lactation. *Osteoporosis Int* 1996;6(suppl 1):283 (abstr).
9. López JM, González G, Reyes V, Campino C, Díaz S. Bone turnover and density in healthy women during breastfeeding and after weaning. *Osteoporosis Int* 1996;6:153–9.

10. Kalkwarf HJ, Specker BL, Bianchi DC, Ranz J, Ho M. The effect of calcium supplementation on bone density during lactation and after weaning. *N Engl J Med* 1997;337:523–8.
11. Prentice A. Calcium supplementation during breast-feeding. *N Engl J Med* 1997;337:558–9.
12. Gruber HE, Gutteridge DH, Baylink DJ. Osteoporosis associated with pregnancy and lactation: bone biopsy and skeletal features in three patients. *Metab Bone Dis Relat Res* 1984;5:159–65.
13. Lissner L, Bengtsson C, Hansson T. Bone mineral content in relation to lactation history in pre- and postmenopausal women. *Calcif Tissue Int* 1991;48:319–25.
14. Naylor A, Newman V. Changes in bone density with lactation. *JAMA* 1993;270:2300 (letter).
15. Zhang J, Fortney JA, Feldblum PL. Re: Pregnancy and lactation as determinants of bone mineral density in postmenopausal women. *Am J Epidemiol* 1993;138:1020–1.
16. Blanch J, Pacifici R, Chines A. Pregnancy-associated osteoporosis: report of two cases with long-term bone density follow-up. *J Rheumatol* 1994;33:269–72.
17. Sinigaglia L, Varenna M, Binelli L, Gallazzi M, Calori G, Ranza R. Effect of lactation on postmenopausal bone mineral density of the lumbar spine. *J Reprod Med* 1996;41:439–43.
18. Ferrari S, Rizzoli R, Chevalley T, Slosman D, Eisman JA, Bonjour J-P. Vitamin-D-receptor-gene polymorphisms and change in lumbar-spine bone mineral density. *Lancet* 1995;345:423–4.
19. Krall EA, Parry P, Lichter JB, Dawson-Hughes B. Vitamin D receptor alleles and rates of bone loss: influence of years since menopause and calcium intake. *J Bone Miner Res* 1995;10:978–84.
20. Labbok M, Krasovec K. Towards consistency in breastfeeding definitions. *Stud Fam Plann* 1990;21:226–30.
21. Prentice A, Parsons TJ, Cole TJ. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. *Am J Clin Nutr* 1994;60:837–42.
22. Coward WA, Cole TJ, Sawyer MB, Prentice AM, Orr-Ewing AK. Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. *Hum Nutr Clin Nutr* 1982;36C:141–8.
23. Coward WA. Measuring milk intake in breast-fed babies. *J Pediatr Gastroenterol Nutr* 1984;3:275–9.
24. Laskey MA, Dibba B, Prentice A. A semi-automated micromethod for the determination of calcium and phosphorus in human milk. *Ann Clin Biochem* 1991;28:49–54.
25. Nelson M, Hague GF, Cooper C, Bunker VW. Calcium intake in the elderly: validation of a questionnaire. *J Hum Nutr Diet* 1988;1:115–27.
26. Braddon FEM, Wadsworth MEJ, Davies JMC, Cripps HA. Social and regional differences in food and alcohol consumption and their measurement in a national birth cohort. *J Epidemiol Community Health* 1988;42:341–9.
27. Paul AA, Southgate DAT. McCance and Widdowson's the composition of foods. 4th ed. London: Her Majesty's Stationery Office, 1978.
28. Schofield WN, Schofield C, James WPT. Basal metabolic rate: review and prediction, together with annotated bibliography of source material. *Hum Nutr Clin Nutr* 1985;39C:1–96.
29. Black AE, Goldberg GR, Jebb SA, Livingstone MBE, Cole TJ, Prentice AM. Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr* 1991;45:583–99.
30. Goldberg G, Black AE, Jebb SA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* 1991;45:569–81.
31. Goldberg GR, Prentice AM, Coward WA, et al. Longitudinal assessment of the components of energy balance in well-nourished lactating women. *Am J Clin Nutr* 1991;54:788–98.
32. Morrison MA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994;367:284–7.
33. Prentice A, Laskey MA, Shaw J, Cole TJ, Fraser DR. Bone mineral content of Gambian and British children aged 0–36 months. *Bone Miner* 1990;10:211–24.
34. Riggs BL, Nguyen TV, Melton LJ, et al. The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. *J Bone Miner Res* 1995;10:991–6.
35. Kent GN, Price RI, Gutteridge DH, et al. Site-specific reduction in bone loss by calcium supplements in normal lactation. *Osteoporosis Int* 1995;5:315 (abstr).
36. Rico H, Revilla M. Factors affecting bone mass. *JAMA* 1994;272:1003–4.
37. Pouillès JM, Trémollières F, Ribot C. Variability of vertebral and femoral postmenopausal bone loss: a longitudinal study. *Osteoporosis Int* 1996;6:320–4.
38. Black AE, Wiles SJ, Paul AA. The nutrient intakes of pregnant and lactating mothers of good socio-economic status in Cambridge, UK: some implications for recommended daily allowances of minor nutrients. *Br J Nutr* 1986;56:59–72.
39. Prentice A, Laskey MA, Jarjou LMA, ed. Lactation and bone development: implications for calcium requirements of infants and lactating mothers. In: Tsang RC, Bonjour JP, eds. Nutrition and bone development. New York: Raven Press (in press).
40. Prentice A, Jarjou LMA, Cole TJ, Stirling DM, Dibba B, Fairweather-Tait S. Calcium requirements of lactating Gambian mothers: effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. *Am J Clin Nutr* 1995;62:58–67.
41. Chan GM, McMurry M, Westover K, Engelbert-Fenton K, Thomas MR. Effects of increased dietary calcium intake upon the calcium and bone mineral status of lactating adolescent and adult women. *Am J Clin Nutr* 1987;46:319–23.
42. Compston JE, Laskey MA, Croucher PI, Coxon A, Kreitzman S. Effect of diet-induced weight loss on total body bone mass. *Clin Sci* 1992;82:429–32.
43. Sowers MF, Eyre D, Hollis BW, et al. Biochemical markers of bone turnover in lactating and nonlactating postpartum women. *J Clin Endocrinol Metab* 1995;80:2210–6.
44. Yamaga A, Taga M, Minaguchi H, Sato K. Changes in bone mass as determined by ultrasound and biochemical markers of bone turnover during pregnancy and puerperium: a longitudinal study. *J Clin Endocrinol Metab* 1996;81:752–6.
45. Dobnig H, Kainer F, Stepan V, et al. Elevated parathyroid hormone-related peptide levels after human gestation: relationship to changes in bone and mineral metabolism. *J Clin Endocrinol Metab* 1995;80:3699–707.
46. Lippuner K, Zehnder H-J, Casez J-P, Takkinen R, Jaeger P. PTH-related protein is released into the mother's bloodstream during lactation: evidence for beneficial effects on maternal calcium-phosphate metabolism. *J Bone Miner Res* 1996;11:1394–9.
47. Sowers MF, Hollis BW, Shapiro B, et al. Elevated parathyroid-related peptide associated with lactation and bone density loss. *JAMA* 1996;276:549–54.