Bone mineral density changes during lactation: maternal, dietary, and biochemical correlates1-3

Nancy F Krebs, Carol J Reidinger, Alastair D Robertson, and Manon Brenner

ABSTRACT  The objectives of this study were to characterize the effects of lactation and weaning on maternal bone mineral density (BMD) and on biochemical markers of bone turnover, and to determine the effects of dietary intake, milk output, and other maternal factors on changes in BMD. Twenty-six fully lactating and eight nonlactating women were followed longitudinally through 7 mo postpartum; the lactating women were followed through postweaning. Maternal dietary and supplement intake data, infant milk intake measurements, blood and urine samples, and midradius and L2-L4 vertebral BMD measurements were obtained 0.5, 3, 5, and 7 mo postpartum. Biochemical analyses included measurements of calciotropic hormones, 24-h urinary excretion of calcium, markers of bone formation and resorption, estradiol, and prolactin. Estimated maternal demands for calcium excretion in milk were met by a combination of high calcium intake (from diet and supplements, 1500 ± 460 mg/d at 0.5 mo for lactating women) and a decline of ~4% in vertebral BMD between 0.5 and 3 mo postpartum. Postweaning BMD (n = 15) at this site approximated initial values. Two factors were positively associated with vertebral BMD, estradiol (P < 0.001) and calcium intake (P = 0.03), whereas two factors were negatively associated, parity (P = 0.03) and protein intake (P = 0.01). In these well-nourished women, the results suggest that the extent of bone loss associated with lactation and its recovery postweaning are negatively influenced by parity. The results also suggest that the bone loss may be attenuated by a generous dietary ratio of calcium to protein. Am J Clin Nutr 1997;65:1738-46.

KEY WORDS  Lactation, bone mineral density, osteoporosis, calcium, protein intake, parity, breast-feeding, postpartum, calciotropic hormones, women

INTRODUCTION

Lactation is a very demanding process in terms of nutritional requirements. Efficient maternal adaptation must occur to successfully compensate for the energy and nutrient requirements of milk production and secretion. Although theoretically a woman could meet the increased nutritional demands solely by increasing her dietary intake, in reality this is not the only adaptive response. For example, the energy cost of lactation is generally met by a combination of increased energy intake and mobilization of fat stores laid down during pregnancy. For any given nutrient, meeting the increased need to support lactation is likely to involve a combination of adaptive responses (1). The present study examined several aspects of maternal calcium nutrition economy during lactation. Although the effect of lactation on the maternal skeleton has been an area of past uncertainty (2-4), several recent studies have shown a significant decline in trabecular bone mineral density (BMD) during lactation (5-9). In addition to the effect on maternal nutritional status in lactation, such observations are of public health interest because of the potential implications for risk of osteoporosis later in life. Peak bone mass is achieved during the childbearing years and is an important factor in reducing the long-term risk of postmenopausal osteoporotic fractures. Thus, identification of factors that influence the extent of bone loss during lactation is important. Earlier studies have been combinations of cross-sectional and longitudinal design, have not controlled for extent of lactation, have had limited dietary and biochemical data to accompany that of the BMD changes, or all of these.

The objectives of this study were to longitudinally characterize the effects of lactation and weaning on BMD and on biochemical markers of bone turnover and calcium homeostasis, and to determine the effects of dietary intake and milk output on changes in BMD during lactation.

SUBJECTS AND METHODS

Study design

Samples and measurements were obtained 0.5, 3, 5, and 7 mo postpartum (± 1 wk at each cycle) and at postweaning, defined as 6 mo after resumption of menses. These included a 3-d maternal diet record, 3-d infant test weighing to determine milk intake, maternal blood and urine samples, and BMD measurements. Biochemical analyses included measurements of the calciotropic hormones 25-hydroxyvitamin D (calcidiol),

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1,25-dihydroxyvitamin D (calcitriol), intact parathyroid hormone (iPTH), and calcitonin; the hormones estradiol and prolactin; markers of bone formation and resorption—osteocalcin and urinary excretion of pyridinoline cross-links, respectively; and major mineral concentrations in serum and 24-h excretions.

Subjects
The women were identified before or shortly after delivery and, if they intended to breast-feed exclusively for ≥ 5 mo and to continue breast-feeding through ≥ 7 mo postpartum, they were enrolled within 2 wk postpartum. Twenty-six lactating subjects and eight postpartum nonlactating control subjects were enrolled over 4 y (1987–1991), with enrollment equally distributed over the calendar year. All subjects were healthy nonsmokers who consumed fewer than two drinks of alcohol per week. The study design and protocol were approved by the Colorado Multiple Institutional Review Board, and informed consent was obtained from all subjects before enrollment.

Dietary intake
Three-day diet records were completed and reviewed for completeness at each of the four data collection times during lactation. Use of dietary supplements was also monitored at each visit. Nutrient analyses were performed by a research nutritionist using the NUTRIPRACTOR software package (Practocare, Inc, San Diego).

Sample collection and analyses
Blood specimens were obtained in the subject’s home in the morning after an overnight fast and ≈2 h after the beginning of the last breast-feeding episode. Specimens were drawn with a syringe and immediately transferred to appropriate Vacutainer tubes (Becton Dickinson, Rutherford, NJ). Separation of plasma or serum was performed within 30 or 60 min, respectively, and samples were stored at −20 °C until analyzed. A 24-h urine collection was obtained from each subject at 1 mo and at each subsequent visit. Mineral-free plastic containers were provided to each subject.

Serum analyses were performed by using immunoradiometric assays for prolactin (IRMA-Count; Diagnostic Products Corp, Los Angeles) and iPTH (N-tact PTH IRMA; Incstar Corp, Stillwater, MN). Radioimmunoassays were used to measure calcitonin (Calcitonin II; Incstar Corp), estradiol (Coat-ACount; Diagnostic Products Corp), osteocalcin (Incstar Corp), and calcitriol and calcidiol (Incstar Corp). Serum and urine assays for creatinine, calcium, phosphorus, magnesium, and serum alkaline phosphatase were run on a Boehringer Mannheim Corp/Hitachi system (Mannheim, Germany) in the hospital clinical laboratory. Calcium and magnesium excretion corrected for glomerular filtration rate were calculated by using the following equation: urine [Ca or Mg] × serum [creatinine]/urine [creatinine], expressed as μmol/L, glomerular filtrate. Tubular reabsorption of phosphorus (TRP) was calculated as 1 − (urine [P] × serum [creatinine]/serum [P] × urine [creatinine]). Urinary excretion of pyridinoline cross-links was measured with an enzyme immunoassay (Metra Biosystems Inc, Palo Alto, CA). All biochemical analyses were run in batches so that all samples for an individual subject were processed at the same time.

Test weighing
Infant milk intakes were measured by weighing the infant before and after all feedings for 3 consecutive days at each of the four data collection times during lactation. Electronic digital balance scales (Sartorius Corp, Bohemia, NY), which integrate 100 rapid serial measurements and provide a mean weight to the nearest 1 g, were set up in the subject’s home for each test-weighing period. Detailed written log sheets with weights and comments were kept by the mothers. These were reviewed by a member of the research team after each test-weighing period. Twenty-four–hour milk intakes were calculated by determining the total intake for the 3-d period, dividing by the exact number of hours, and multiplying by 24.

Bone densitometry measurements
Dual-photon absorptiometry (DPA) (DP3; Norland, Madison, WI) was used for measurement of spinal BMD, reporting BMD at the anteroposterior L2–4 region, a sensitive measurement of trabecular bone. A single-photon densitometer (model 278; Norland) was used to measure BMD of the proximal or midthird radius, almost exclusively cortical bone. Approximately midway through the study, the Radiology Department upgraded to dual-energy X-ray absorptiometry (DXA). DXA (DPX; Lunar Corp, Madison, WI) was used for all subsequent measurements of the spine. Twenty patients were scanned with both DPA and DXA as the DXA machine was being installed to check the correlation between the machines using the latest available software for each and a gadolinium source that was <1 mo old for the DP3. There was a highly significant correlation between the techniques (r = 0.98), but a small, consistent decrease in BMD measurements with DXA compared with DP3, as reported previously in comparison studies (10, 11). Fortunately, the Lunar system software allowed rebuilding of the DP3 database into the DXA machine; we were therefore able to reanalyze all previous measurements done by DPA with the DPX software using the newer and more precise edge-detection algorithm. Each set of four data points for each subject was therefore analyzed consistently by the same bone-edge detection factor. Reproducibility measurements of the spine by our technicians were improved from 2% with the DP3 to 1% with the DPX.

Ten women had all four of their measurements taken by DPA. Eleven women had all of their measurements taken by DXA. Fourteen women had one to three measurements taken by DPA, and subsequent scans by DXA. These 14 subjects had all four data sets analyzed as discussed above. There was no alteration in the pattern of BMD loss and recovery for these subjects and they are included in the analysis.

Data analysis
Data management and graphic and statistical analyses were performed with SAS version 6.07 (SAS Institute, Inc, Cary, NC). Longitudinal data were plotted extensively as a guide to statistical analysis. The principle statistical procedure used was the general linear model, including repeated-measures analysis of variance (ANOVA), regression, and r test. Biochemical variables, dietary intake variables, and BMD were analyzed separately as functions of cycle (both linear and quadratic), group (lactating and nonlactating), and the interaction of cycle and group; interaction refers to the difference in the effect of
the cycle between groups. Unless otherwise indicated, data are presented as means ± SDs.

Stepwise regression with backward elimination was performed with vertebral (L2-L4) BMD and the slope of the change between 0.5 and 3 mo used separately as dependent variables. Independent variables were maternal characteristics (age, parity, height, weight at each cycle, and body mass index), maternal dietary intake (energy, protein, dietary and total calcium, the ratio of calcium to protein, phosphorus, and supplemental vitamin D), infant milk intake, and estradiol and prolactin concentrations.

RESULTS

Descriptive data for the subjects in both groups are given in Table 1. The date of resumption of menses was available for 19 lactating subjects, for whom the mean was 8.7 ± 4.1 mo. Fifteen of the lactating women completed the postweaning measurements, which were obtained on average at 15 mo postpartum (range: 10–26 mo). The lactating women were all white and two of the eight nonlactating women were African American. Oral contraceptives (low-estrogen type) were used during the study period by one lactating subject (starting before the 2-wk measurements) and two nonlactating subjects (starting at 2 and 3 mo). Maternal weights for the lactating subjects declined significantly over the course of lactation (P < 0.001); means were 63.3 ± 9.2 and 61.0 ± 9.6 kg at 0.5 and 7 mo, respectively.

A summary of the calculated dietary intakes of energy, protein, calcium, and phosphorus at each cycle through 7 mo postpartum for both groups is given in Table 2; intakes from supplements of calcium and vitamin D are also indicated. Mean intakes of protein were well above the recommended dietary allowance (RDA) (12) for both groups, ranging from 140% to 153% for the lactating women. Dietary calcium intake was > 90% of the RDA for both groups at all time points; with the addition of calcium from supplements, the total intake exceeded the RDA. The mean ratio of dietary calcium to protein ranged from 12 to 14 for the lactating women and from 11 to 13 for the nonlactating women. With the addition of the calcium supplements, the ratios increased to 14–16 throughout the study period for the lactating women. For the nonlactating women, the mean ratios of calcium to protein from diet alone ranged from 11 to 13 and were not substantially changed by supplement use.

<table>
<thead>
<tr>
<th>TABLE 1 Characteristics of study subjects</th>
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<td>Lactating (n = 26)</td>
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<tr>
<td>Age (y)</td>
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<td>Parity</td>
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<td>Postpartum weight (kg)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Infant birth weight (kg)</td>
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</table>

1 ± SD; n in brackets.

2 Duration of lactation for previous child in subjects with parity > 1.

Longitudinal concentrations of calcitropic hormones are shown in Figure 1. Mean iPTH concentrations were consistently lower in the lactating women but the group effect was only marginally significant and time effects were not significant. Both indexes of vitamin D status showed significant time effects: calcidiol declined over the postpartum period whereas calcitriol increased in both groups. Although the mean concentrations of calcidiol were consistently lower in the lactating women than in the nonlactating women, the difference was not significant (P = 0.08). Concentrations of calcitonin declined slightly over time postpartum but also did not differ significantly by group. Because subjects in both groups were enrolled throughout the calendar year, seasonal effects were unlikely.

Estradiol concentrations (Figure 2) were significantly lower in the lactating women, with up to a 3.5-fold difference between group means at 3 and 5 mo postpartum. The mean concentration for the lactating women postweaning was virtually the same as that for the nonlactating women 7 mo postpartum. Serum prolactin concentrations were severalfold higher in the lactating women at all time points during lactation and steadily declined over the 7 mo of lactation.

Serum osteocalcin concentrations (Figure 3) increased between 0.5 and 3 mo in the lactating women and remained higher than those of the nonlactating women until the postweaning measurement. Urinary excretion of pyridinoline crosslinks (Figure 3) declined sharply in the early weeks postpartum and then more gradually over time; concentrations did not differ significantly by group, although there was a trend for higher excretion by the lactating subjects at 1 and 3 mo (P = 0.14 and 0.07, respectively).

Mean serum concentrations of calcium, phosphorus, and magnesium were within normal limits at all time points, did not change significantly over time, and were similar between groups (Table 3). Urinary calcium excretion, though consistently higher in the lactating women, did not differ significantly by time or group. Absolute urinary excretion of magnesium and phosphorus was significantly higher for the lactating women but no time effects were observed for either group. Magnesium excretion corrected for glomerular filtration rate was not significantly different between groups. TRP means were significantly lower in the lactating group and had increased at the postweaning measurement.

Mean 24-h milk outputs at 0.5, 3, 5, and 7 mo postpartum were 600 ± 135, 700 ± 100, 720 ± 130, and 650 ± 150 g/d, respectively, for the lactating subjects. Initial postpartum BMD was not significantly different between the two groups at either of the sites measured and was not related significantly to maternal age, height, weight, or body mass index. Maternal weight and L2-L4 BMD over the entire study period were weakly correlated (r = 0.17, P = 0.13). BMD at the midradius did not change significantly over the study period for either group. The density at the L2-L4 vertebral site, however, declined by 0.049 ± 0.072 g/cm² between 0.5 and 3 mo for the lactating subjects (Figure 4). At the postweaning measurement, the mean BMD at the vertebral site was not significantly different from the 0.5-mo measurement. Regression analysis indicated a significant quadratic effect of time for the lactating women (P < 0.001) but not for the nonlactating women (P = 0.98).

Parity, examined as a continuous variable, was negatively associated with vertebral BMD at 0.5 and 3 mo (P = 0.02 and
0.03, respectively) and marginally so at 7 mo (P = 0.06) and at the postweaning measurement (P = 0.08). When cycle and parity were used as independent variables in regression with vertebral BMD, parity remained significant (P = 0.03).

Stepwise regression indicated four factors that were associated significantly with L2-L4 BMD in the lactating subjects. Estradiol (P < 0.001) and calcium intake, both dietary and total (P = 0.03), were positively associated, whereas parity (P = 0.03) and dietary protein (P = 0.01) were negatively associated. When the ratio of total dietary calcium to protein was added to the stepwise analysis, calcium and protein dropped out as individual variables and the ratio was positively associated (P = 0.01). Because of the small number of subjects with parity > 2 (n = 3), and to avoid an undue weighting of the data from these subjects, parity was also considered as 1 versus > 1 (n = 12 and 13, respectively), with similar results.

In view of the results of the stepwise analyses, other selected relations were examined. The dietary protein intake of the lactating subjects was weakly correlated with 24-h urine calcium excretion at 7 mo postpartum (r = 0.35, P = 0.09). Estradiol concentrations were negatively correlated with 24-h milk output (P < 0.01, ANOVA) and L2-L4 BMD was also negatively associated with milk output (P = 0.01).

**DISCUSSION**

Of the potential means of maternal adaptation to meet the demands of lactation for calcium secretion into milk, we investigated dietary intake, urinary excretion, mean daily infant milk intake (as a surrogate for milk output), and bone mobilization. Various biochemical indexes were also monitored to provide insight into the mechanisms of adaptation. The results of this study are, to our knowledge, the first that relate longitudinal data on dietary intake, milk output, and biochemical markers to changes in BMD through lactation and the weaning process.

Although the actual daily calcium output in milk was not measured, calcium concentrations in milk are generally considered to be independent of maternal dietary intake, at least if maternal calcium intake approximates current recommendations (13, 14). When the longitudinal data for milk calcium concentrations (13) were applied to the test-weighing figures from the present study, estimates of the calcium output in milk were 200 mg/d at 0.5 mo and between 170 and 190 mg/d from 3 to 7 mo. This represents a decline of ~15% in the daily calcium loss in the milk between early and later lactation and thus in the demands for maternal adaptation.

The mean dietary intakes of energy, protein, and major minerals of these women were generous relative to US dietary standards (12, 15) and were similar to other reports from developed countries (4, 7, 16–18). Calcium intake relative to energy intake was similar for the two groups. Because the lactating women consumed supplemental calcium in greater amounts, however, their overall calcium density was higher at all time points in the postpartum period. Fractional absorption of calcium has not been found to increase during lactation, even in women with low calcium intakes on a short-term basis (19, 20). When a value of 0.20 is used for fractional absorption (19), the gain in absorbed calcium from the increased intake by the lactating women compared with the nonlactating women in this study would be ~100 mg/d at all times evaluated during 7 mo of lactation. In populations with chronically low calcium intakes, the contribution during lactation of adaptation in calcium absorption or excretion of endogenous calcium is unknown (21).
FIGURE 1. Mean (± SEM) longitudinal concentrations of calcitropic hormones for lactating subjects (□) and nonlactating postpartum control subjects (○). Range in parentheses on y axis is the normal range for the laboratory. "Time" refers to linear regression; "time²" refers to quadratic regression.

FIGURE 2. Mean (± SEM) longitudinal concentrations of estradiol and prolactin for lactating subjects (♀) and nonlactating postpartum control subjects (○). Range in parentheses on y axis is the normal range for the laboratory. "Time²" refers to quadratic regression.

The daily urinary excretion of calcium was consistently slightly but not significantly higher in the lactating women. Although renal calcium conservation has been reported in lactating women with lower total calcium intakes (6, 19, 22), our data do not indicate that the kidneys were conserving calcium, which agrees with other observations in lactating women with high mineral intakes (9).

The change in lumbar spine BMD in early lactation represented a decline of ~4%, a figure consistent with other reports (7, 8). If total body calcium in the maternal skeleton is estimated to be 1000–1200 g, 20% of which is trabecular (23), then a 4% decline would provide ~9.6 g Ca. Because the decline in BMD occurred primarily between 2 wk and 3 mo, roughly 130 mg/d would have been available to the exchangeable calcium pool. Thus, the combination of a generous calcium intake and the mobilization of calcium from the maternal skeleton appear to be primarily responsible for meeting the demands for calcium output in milk (~200 mg/d) in these lactating women, particularly during the early months postpartum when calcium output is highest (14).

After the early decline, vertebral BMD remained relatively stable through 7 mo of lactation. In contrast with the early decline in vertebral BMD, no changes were observed in the midradius. This was likely because of the compact nature of the bone in this area. In those subjects who completed postweaning measurements, the mean BMD had returned to values not significantly different from those at 2 wk postpartum, suggest-
ing, as have others (6–8), that the decline in BMD associated with lactation is temporary. Note that although the means at 2 wk and postweaning were not significantly different, those at postweaning were lower. Sowers et al (7) also observed that, for women still lactating at 9 mo, the BMD of the lumbar spine was still significantly lower than baseline measurements at 2 wk (7). In this and other studies, prepregnancy measurements have not been obtained, raising the possibility that the 2-wk baseline value is actually elevated because of accretion during the high estrogen state of pregnancy. The negative association of BMD and parity argues against this and raises the possibility of incomplete “catch-up,” depending on the frequency and spacing of pregnancy and lactation periods. The small range of parity for the majority of subjects and the relatively small number of women with parity > 2 make definitive conclusions about the effect of parity and repeated cycles of pregnancy and lactation difficult to determine from this study. When parity was considered as 1 versus > 1, however, the negative effect remained significant, suggesting that there may be less than complete restoration of BMD. All but one of the lactating subjects with parity > 1 had breast-fed previously, most for several months (Table 1). This observation needs further examination by longitudinal studies including a larger number of women after more than one cycle of extended lactation. Of some interest was the finding that the lowest vertebral BMD observed in the study at all points was that of a woman with parity of 5 and in her fifth lactation cycle. She also, however, had a family history of osteoporosis.

The longitudinal biochemical data in these women provide further insight into the mechanisms of calcium homeostasis in lactation. Of the classic calcitropic hormones, iPTH was consistently lower in the lactating subjects, suggesting that an increased release of this hormone was not a primary mechanism responsible for maintaining calcium concentrations in serum and milk. The pattern of relatively high urinary calcium excretion in the lactating women was consistent with the low iPTH concentrations. Similar findings were suggested in cross-sectional studies of postpartum women (6, 17, 24, 25) and by one longitudinal study (26), all of which were also conducted in well-nourished populations. In contrast with other reports with postweaning data (6, 9), we observed neither an elevation in iPTH concentrations nor reduced calcium excretion at the postweaning measurement. This discrepancy may be related to differences in timing or definition of the postweaning sampling or in dietary intake and nutritional status.

Relatively high circulating concentrations of PTH-related protein (PTHrP), the factor thought to be responsible for hypercalcemia of malignancy and proposed to exert local effects in mammary tissue (such as mediating calcium transport into milk), were reported in some studies of lactating women (24). Although PTHrP was not measured in this study, the biochemical data do not strongly suggest an effect, although the lower TRP values in the lactating women would be consistent. More comprehensive data will be necessary to clarify its role in lactation.

Neither of the indexes of vitamin D differed significantly between the lactating and nonlactating women. Increasing calcitriol concentrations over the course of lactation have been suggested to reflect more marginal calcium status with prolonged lactation (26, 27). When interpreted in conjunction with the PTH data, the declining milk calcium output (14), and the stabilization of BMD, however, this explanation seems unlikely in this group of women. In these lactating subjects with generous intakes of both calcium and vitamin D, the patterns of the vitamin D indexes do not suggest a role in the maintenance of calcium homeostasis in early lactation. The significant increase in calcitriol concentrations may indicate an increase in calcium absorption in later lactation when estradiol concentrations have begun to rise and bone resorption has slowed, but supportive experimental data are lacking. Calcium supplementation of well-nourished lactating women also was not associated with significant mean differences in either of the vitamin D indexes, although longitudinal data during lactation were not reported (9). Data from lactating women with much lower dietary calcium intakes suggest that stimulation of these homeostatic responses occurs under more marginal nutritional circumstances (13).

Estradiol concentrations were strongly related to BMD. Estrogen has potent effects—it stimulates osteoblastic proliferation and enhances collagen gene expression, and hypoestrogenemic states are associated with increased bone resorption (23). The relative hypoestrogenemia of lactation is likely to play at least a permissive role in the bone mobilization observed. Milk output and estradiol concentrations were negatively associated in these subjects, all of whom breast-fed exclusively through at least the first 5 mo postpartum. One recent study, using return of menses as an indicator of estrogen status, also found a larger bone loss associated with more prolonged lactational amenorrhea (8). The largest decline in BMD in the present study...
TABLE 3
Serum concentrations and daily excretion of major minerals in lactating (n = 26) and nonlactating (n = 8) groups

<table>
<thead>
<tr>
<th></th>
<th>0.5 mo</th>
<th>1 mo</th>
<th>3 mo</th>
<th>5 mo</th>
<th>7 mo</th>
<th>Postweaning</th>
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<tr>
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<tr>
<td>Lactating</td>
<td>2.4 ± 0.1</td>
<td>—</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.1</td>
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<tr>
<td>Nonlactating</td>
<td>2.4 ± 0.1</td>
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<td>2.4 ± 0.1</td>
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<td>2.4 ± 0.2</td>
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<td>Phosphorus (mmol/L)</td>
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<tr>
<td>Lactating</td>
<td>1.2 ± 0.1</td>
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<td>1.3 ± 0.2</td>
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<td>1.2 ± 0.2</td>
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<tr>
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<td>1.2 ± 0.1</td>
<td>—</td>
<td>1.1 ± 0.2</td>
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<td>1.2 ± 0.2</td>
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<td>Magnesium (mmol/L)</td>
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<tr>
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<td>0.8 ± 0.1</td>
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<tr>
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<td>0.8 ± 0.1</td>
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<td>0.8 ± 0.1</td>
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<td>Urinary excretion</td>
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<tr>
<td>Lactating</td>
<td>—</td>
<td>2.1 ± 1.4</td>
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<td>1.9 ± 1.4</td>
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<tr>
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<td>—</td>
<td>16 ± 9</td>
<td>27 ± 21</td>
<td>20 ± 10</td>
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<td>0.86 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td>Nonlactating</td>
<td>0.87 ± 0.07</td>
<td>0.86 ± 0.05</td>
<td>0.90 ± 0.04</td>
<td>0.88 ± 0.04</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Magnesium (mmol/d)^4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating</td>
<td>4.6 ± 1.8</td>
<td>3.9 ± 1.3</td>
<td>4.4 ± 2.0</td>
<td>4.4 ± 1.9</td>
<td>4.0 ± 1.5</td>
<td>—</td>
</tr>
<tr>
<td>Nonlactating</td>
<td>3.4 ± 1.9</td>
<td>3.1 ± 0.8</td>
<td>2.5 ± 1.4</td>
<td>3.2 ± 1.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(μmol/L)^2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactating</td>
<td>47 ± 49</td>
<td>31 ± 10</td>
<td>33 ± 14</td>
<td>33 ± 12</td>
<td>28 ± 8</td>
<td>—</td>
</tr>
<tr>
<td>Nonlactating</td>
<td>32 ± 27</td>
<td>23 ± 9</td>
<td>21 ± 6</td>
<td>29 ± 8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 ± SD
2 Excretion corrected for glomerular filtration.
3 Significant group effect, P < 0.01.

Prolactin has been proposed to stimulate bone mobilization, possibly by altering estrogen secretion, by stimulating calcitriol, or through more direct effects (25, 28, 29). Although the mean concentration of prolactin at 3 mo was approximately half that at 2 wk, concentrations remained above the normal range throughout lactation. The persistent elevation of prolactin concentrations throughout lactation in conjunction with low estradiol concentrations may provide a synergistic milieu promoting bone demineralization. Unlike estradiol, however, prolactin was not related significantly to BMD changes, suggesting a less direct or potent effect.

Markers of bone turnover were higher in the lactating women. Only osteocalcin, a marker of osteoblastic activity, differed significantly between the two groups and was generally higher in the lactating women; however, at 2 wk postpartum the mean was slightly lower than that of the nonlactating women. This finding differs from that of other reports in which osteocalcin was significantly higher (30) or the same (7) in lactating women at a similar stage of early lactation, although the former results were from women who had delivered prematurely (30). Other investigators have reported results during established lactation similar to those in the current study, ie, higher mean concentrations in the lactating women (7, 17, 30).

FIGURE 4. Mean (± SEM) longitudinal vertebral bone mineral density (BMD) measurements of the L2-L4 spine in lactating (●) and nonlactating postpartum control (○) subjects. "Time" refers to quadratic regression; the P value applies to the lactating subjects only.
In contrast, the excretion of pyridinoline cross-links, a marker for bone resorption, fell precipitously for both groups from 2 wk to 1 mo and was slightly higher in the lactating women at the 1- and 3-mo visits. It is possible that the relatively small number of nonlactating women provided insufficient power to detect a true difference between the groups for this marker in the early months. Excretion remained virtually the same between groups thereafter, coincident with the relatively stable BMD from 3 to 7 mo in the lactating women.

To assess the factors associated with the bone loss during lactation, a selected number of variables were examined in the multivariate analyses. By far the strongest factor explaining the variance was intersubject variability, supportive of the recognized importance of genetic factors in determining BMD (31). Beyond this, parity > 1 was associated with a ~10% lower BMD. Estradiol concentrations, although strongly associated with BMD, accounted for a much smaller portion of the overall variance.

A positive association between the ratio of calcium to protein and vertebral BMD has not been reported previously in lactating women and represents a potentially modifiable factor. Sowers et al (7) found no relation with estimated dietary calcium, whereas Chan et al (4) reported a positive correlation between calcium intake and BMD in adolescents. More recently, calcium supplementation during lactation that brought intakes to > 2 g/d did not prevent spinal bone loss but was associated with an increase in BMD at the ultradistal radius (9). In nonlactating young women, the ratio of calcium to protein has been reported to be the most important determinant of the rate of bone gain (32). It is likely that the effect of protein intake was at least partially related to increased urinary excretion of calcium, given the suggestive correlation, and possibly also with some increase in fecal calcium (33). Phosphorus and vitamin D intakes, which also influence calcium homeostasis, were not significantly associated with BMD in the present study. These dietary data suggest that for lactating women there may be some disadvantage of relatively high protein intakes from sources not also rich in calcium. In populations with low calcium intakes (21), the effects of lactation on BMD may be attenuated by a low-protein diet. In women who do not consume dairy products, which have particularly high ratios of calcium to protein, use of calcium supplements may be prudent if the diet is otherwise high in protein.

The results of this study support previous observations of bone loss associated with extended lactation, with subsequent recovery of BMD postweaning. In addition, dietary data suggest factors that may attenuate or exacerbate the changes in BMD. Successfully breast-feeding an infant is dependent on generous milk output and thus seems inevitably linked to a prolonged period of relative hypoestrogenemia and thus to bone resorption. The findings of this study provide further general reassurance that bone resorption is reversed, but the negative association of parity with BMD raises the possibility of some persistent effect on BMD. The results of this study should provide additional data from which health care providers may base dietary recommendations for lactating women.

We thank the women who participated in this study for their remarkable dedication and conscientiousness, the Medela and Ameda/Egnell companies for loaning electric breast pumps to the participating mothers, Susan Hartley for dietary analyses, Jamie Westcott for supervision of laboratory analyses, and Janet King and K Michael Hambridge for their intellectual contributions.

REFERENCES