

# A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses<sup>1-3</sup>

Lorrene D Ritchie, Ellen B Fung, Bernard P Halloran, Judith R Turnlund, Marta D Van Loan, Christopher E Cann, and Janet C King

See corresponding editorial on page 591.

**ABSTRACT** To clarify the role of the intestine, kidney, and bone in maintaining calcium homeostasis during pregnancy and lactation and after the resumption of menses, a longitudinal comparison was undertaken of 14 well-nourished women consuming  $\approx 1200$  mg Ca/d. Measurements were made before conception (prepregnancy), once during each trimester of pregnancy (T1, T2, and T3), early in lactation at 2 mo postpartum (EL), and 5 mo after resumption of menses. Intestinal calcium absorption was determined from the enrichment of the first 24-h urine sample collected after administration of stable calcium isotopes. Bone mineral of the total body and lumbar spine was measured by dual-energy X-ray absorptiometry and quantitative computerized tomography, respectively. Twenty-four-hour urine and fasting serum samples were analyzed for calcium, calcitropic hormones, and biochemical markers of bone turnover. Despite an increase in calcium intake during pregnancy, true percentage absorption of calcium increased from  $32.9 \pm 9.1\%$  at prepregnancy to  $49.9 \pm 10.2\%$  at T2 and  $53.8 \pm 11.3\%$  at T3 ( $P < 0.001$ ). Urinary calcium increased from  $4.32 \pm 2.20$  mmol/d at prepregnancy to  $6.21 \pm 3.72$  mmol/d at T3 ( $P < 0.001$ ), but only minor changes in maternal bone mineral were detected. At EL, dietary calcium and calcium absorption were not significantly different from that at prepregnancy, but urinary calcium decreased to  $1.87 \pm 1.22$  mmol/d ( $P < 0.001$ ) and trabecular bone mineral density of the spine decreased to  $147.7 \pm 21.2$  mg/cm<sup>3</sup> from  $162.9 \pm 25.0$  mg/cm<sup>3</sup> at prepregnancy ( $P < 0.001$ ). Calcium absorption post-menses increased nonsignificantly to  $36.0 \pm 8.1\%$  whereas urinary calcium decreased to  $2.72 \pm 1.52$  mmol/d ( $P < 0.001$ ). We concluded that fetal calcium demand was met by increased maternal intestinal absorption; early breast-milk calcium was provided by maternal renal calcium conservation and loss of spinal trabecular bone, a loss that was recovered postmenses. *Am J Clin Nutr* 1998;67:693-701.

**KEY WORDS** Pregnancy, lactation, postmenses, dietary calcium, calcium absorption, urinary calcium, bone mineral, bone turnover, calcitropic hormones, breast-milk calcium, women

## INTRODUCTION

The physiologic demand for calcium is elevated by as much as 200-300 mg/d during pregnancy and lactation (1, 2). This

demand can theoretically be satisfied by an increase in dietary calcium, an increase in intestinal calcium absorption, a decrease in urinary calcium excretion, or mobilization of maternal bone mineral. Past human studies, most of which were cross-sectional, are inconclusive as to which homeostatic responses occur (for reviews, see references 3 and 4). Recent studies have found that compared with absorption in the nonpregnant state, calcium absorption is elevated during pregnancy and after weaning but not during lactation. Daily urinary calcium excretion has been reported to increase during pregnancy and decrease or be similar to that at prepregnancy during lactation. Limited data suggest a conservation of renal calcium after weaning. Retrospective studies of the relation of parity, history of breast-feeding, or both with maternal bone mineral density (BMD) of the spine have had conflicting results. Many but not all prospective studies have shown that pregnancy, lactation, or both result in maternal loss of spinal bone mineral. The relatively few studies to include postweaning women have shown recovery of spinal bone. Little is known about the response of total-body bone mineral to pregnancy, lactation, or the resumption of menses.

The objective of this study was to examine prospectively the effect of pregnancy, lactation, and the resumption of menses on 1) intestinal calcium absorption, 2) urinary calcium, 3) spinal trabecular and total-body bone mineral, and 4) serum calcium, calcitropic hormones, and biochemical markers of bone turnover. This study is unique in that it is the first to include longitudinal

<sup>1</sup> From the Department of Nutritional Sciences, University of California, Berkeley; the Department of Medicine, VA Medical Center, San Francisco; the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Presidio of San Francisco; and the Department of Radiology, University of California, San Francisco.

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<sup>3</sup> Address reprint requests to JC King, US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, PO Box 29997, Presidio of San Francisco, CA 94129-0997.

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measurements of all major aspects of calcium homeostasis in the same women, beginning before conception and continuing through lactation and after the resumption of menses postpartum.

## SUBJECTS AND METHODS

### Subjects

Subjects were recruited from advertisements posted at the University of California, Berkeley, and placed in local newspapers. Volunteers were screened by interview and excluded from the study based on the following criteria: low calcium intake (< 800 mg/d) as assessed by 24-h recall and frequency of consumption of dairy foods, vegetarian diet, age < 22 y or > 42 y, self-reported body mass index (in kg/m<sup>2</sup>) < 17 or > 27, daily consumption of more than three cups of coffee (or the equivalent in terms of caffeine-containing beverages), cigarette smoking, drug or alcohol abuse, or chronic health problems.

Of the ≈180 women who responded to advertisements, 22 volunteers agreed to participate and were eligible for inclusion in the study, 16 of whom subsequently became pregnant. Two subjects withdrew from participation early in the study: one because of miscarriage and another because of health concerns. Of the remaining 14 subjects, 1 woman conceived before any pregnancy measurements could be scheduled and another 3 women conceived before initial bone measurements were completed. Mean duration between initial measurement and conception was 3.3 ± 3.2 mo. All women were white and from middle-income to upper-middle-income economic strata. Three women had a parity of one and had weaned their previous child a minimum of 2 mo before initial measurements; all other women were nulliparous. All women received prenatal medical supervision beginning in the first trimester and none delivered prematurely or suffered major complications at parturition. The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and informed consent was obtained from all subjects.

### Study design and test-day protocol

Six time points were examined in this longitudinal study: before conception (pregnancy); the first (8–10 wk; T1), second (23–26 wk; T2), and third (34–36 wk; T3) trimesters of pregnancy; during early lactation (6–10 wk postpartum; EL); and after the resumption of menses (5 ± 2 mo postmenses). At each time point the following were assessed: dietary calcium, intestinal calcium absorption, 24-h urinary calcium, and blood and urine chemistry indexes. Bone mineral was measured at pregnancy, 1–2 wk postdelivery, EL, and postmenses. Breast-milk calcium was measured at EL.

Subjects collected a baseline 24-h urine sample in the week preceding the test day. To minimize the influence of dietary variation in these free-living subjects, the day before each test day the subjects consumed a standardized diet containing 8400 kJ and 1100 mg Ca, which was consistent with the composition of their usual diets. Specified foods containing negligible calcium (eg, saltine crackers, soups containing no milk or cream, and fresh fruit) were allowed if additional dietary energy was desired. At each time point, after an overnight fast, subjects consumed <sup>44</sup>Ca (18.9 ± 2.0 mg) dissolved in a nonnutritive beverage (Crystal Lite; Kraft General Foods, White Plains, NY) with a standardized breakfast (toasted English muffin and peanut but-

ter). At all time points except T1, 25 min after the start of breakfast 2 mL <sup>42</sup>Ca (5.9 ± 1.4 mg) was infused over 1–2 min via an indwelling catheter into the antecubital vein, followed by a 5-mL saline rinse. After the infusion, subjects collected a single 24-h urine sample. Three to five days after the test day, an additional fasting blood sample was drawn for the determination of serum calcium, calcitropic hormones, estradiol, and osteocalcin. At T1, a stable-isotope infusion was not performed because of the risk of miscarriage.

### Diet analysis

To minimize the effect of changes in calcium consumption on calcium homeostasis, subjects were instructed to maintain their usual intake of dairy products and other calcium-rich foods for the duration of the study. Subjects also consumed a prenatal multivitamin and mineral supplement every morning containing 160 mg Ca, 125 mg P, and 10 μg vitamin D (Stuart Formula; Johnson & Johnson/Merck, Consumer Pharmaceuticals Company, Fort Washington, PA). To facilitate compliance, subjects used pill dispensers labeled with each day of the week. On advisement of their obstetricians, five subjects took additional daily iron supplements for treatment of anemia during pregnancy and early lactation.

Dietary intakes were assessed from weighed food intake records obtained for 2 weekdays and 1 weekend day during the specimen collection period after each test day. All foods and beverages except water were weighed to the nearest gram on a portable electronic balance (model 1042–22; Cole-Parmer Instrument Company, Chicago). Before being coded for computer entry, diets were reviewed and additional information obtained when necessary. To minimize inconsistencies in diet analysis, all coding was performed by the same individual at the completion of the study. Diet composition was determined by using a computerized database (software version 8.5, NUTRITIONIST III; N-Squared Computing, Salem, OR).

### Urinalysis

To minimize the variation in measurement that might occur over the 3-y study period, samples from the same subject collected at different time points were analyzed together. Urine was collected into 4-L jugs, and aliquots were acidified to a pH of ≈2 by adding 1 mL concentrated trace metal-grade hydrochloric acid (Fisher Scientific, Pittsburgh) per 125 g urine and stored at –20 °C. Total urinary calcium was determined by atomic-absorption spectroscopy (AAS) (model 22; Thermal Jarrell Ash, Franklin, MA) on acidified aliquots after dilution (1:20–1:150) with 0.27% lanthanum chloride (Fisher Scientific) in 0.125 mol HCl/L. A reference urine sample (Lyphochek; Bio-Rad Laboratories, Anaheim, CA) was analyzed simultaneously with each set of samples (CV: 2.2 ± 1.1%). Total daily calcium excretion was determined from the calcium concentration and total urine weight. Collagen cross-links (pyridinoline) were determined on unacidified aliquots with an enzyme-linked immunosorbent assay (Metra Biosystems Inc, Palo Alto, CA).

### Stable-isotope preparation and analysis

Highly enriched stable isotopes, <sup>42</sup>Ca and <sup>44</sup>Ca, were purchased as calcium carbonate from Oak Ridge National Laboratory (Oak Ridge, TN), Cambridge Isotope Laboratories (Woburn, MA), and Atomergic Chemetal Corporation (Farmingdale, NY). Stock solutions of ≈2 g Ca/L were prepared by weighing enriched calcium carbonate to the nearest 0.01 mg, dissolving the calcium carbonate

in a 1.5-fold stoichiometric excess of 1 mol HCl/L, diluting the solution to volume with deionized water, and storing at 4 °C. Stock solutions of  $^{42}\text{Ca}$  were filtered, tested for pyrogens, and bottled into single-dose injection vials at the University of California, San Francisco, pharmacy. The amount of solution administered intravenously was calculated from the weight of the syringe before and after infusion. The day before each test day,  $\approx 10$  mL of the oral  $^{44}\text{Ca}$  stock solution was weighed into a plastic cup and diluted with 250 mL of the nonnutritive beverage. Doses administered were calculated from the total calcium concentration as determined by AAS, the enrichment provided by the isotope supplier, and the weight of the administered dose.

Stable isotopic ratios of prepared urine samples were measured by thermal-ionization mass spectrometry with a magnetic sector mass spectrometer (model 261; Finnigan MAT, San Jose, CA) as described previously (5). Urine samples were prepared by using the calcium oxalate precipitation method (6). For each sample,  $^{42}\text{Ca}$  and  $^{44}\text{Ca}$  were measured relative to  $^{40}\text{Ca}$  and normalized for the natural abundance of  $^{43}\text{Ca}$ : $^{40}\text{Ca}$  to correct for fractionation, and 10 ratio measurements were averaged. The relative SD of measurements was  $\leq 0.2\%$ . True percentage absorption (TPA) of calcium was calculated with the urine ratio method (7) by using the ratio of oral to intravenous calcium stable-isotope enrichments in the first 24-h urine sample after isotope administration. TPA was expressed as a percentage of the dose of stable isotope administered orally.

#### Bone measurements

Volumetric BMD, expressed as  $\text{mg}/\text{cm}^3$ , of trabecular bone for the first and second vertebrae of the lumbar spine was measured by quantitative computerized tomography (model 9800; General Electric, Milwaukee). One dual-energy cross section was scanned to adjust for thickness of bone marrow; bone mineral was then measured by a single-energy scan. Accuracy and precision were 6–9% and 1%, respectively (8). Bone mineral content (BMC; in g) and areal BMD (in  $\text{g}/\text{cm}^2$ ) of integral bone for the total body and component regions, arms, legs, and trunk (which includes the sternum, ribs, pelvis, and spine) were determined by dual-energy X-ray absorptiometry (DXA) (software version 3.6, medium scan time, whole-body scanning mode, model DPX; Lunar Radiation Corporation, Madison, WI). Manufacturer-reported accuracy and precision were 8–10% and 2%, respectively, for BMC measurements, and 4–6% and 1%, respectively, for BMD measurements (9).

#### Breast-milk analyses

At EL, breast-milk volume was determined by the test-weighing procedure (10). Briefly, the baby was weighed to the nearest 0.1 g (model LC34; Sartorius, Dublin, CA) before and after each feeding for 3 d. The daily weight of milk consumed was averaged and adjusted for insensible water losses (11). During a separate 24-h period, complete breast-milk samples were electrically pumped (Lactina; Medela, McHenry, IL) from alternate breasts at each feeding while the woman breast-fed her baby on the opposite breast, in accordance with the alternate-breast-expression method (12). Samples ( $\approx 10$  mL) from each feeding were stored at  $-20^\circ$ ; the remaining milk was offered to the baby at the next feeding. Samples were dry ashed, diluted, and analyzed for calcium by AAS.

#### Blood analyses

All blood samples were collected at  $\approx 0800$  after an overnight fast and at the prepregnancy and postmenses time points in the

middle of the luteal phase of the menstrual cycle. The approximate day of ovulation was determined by a rise in basal temperature (measured orally with a digital thermometer every morning before subjects got out of bed). After collection, blood samples were placed on ice and allowed to sit for a minimum of 1 h before being rimmed and spun at  $1400 \times g$  for 10 min at room temperature in a clinical tabletop centrifuge. Serum aliquots designated for assay of parathyroid hormone (PTH) were stored at  $-80^\circ\text{C}$ ; all others were stored at  $-20^\circ\text{C}$ . Serum concentrations were determined as follows: calcium by AAS, osteocalcin (Immunotopics, San Clemente, CA) and intact PTH (Allegro; Nichols Institute, San Juan Capistrano, CA) by immunoradiometric assay, calcitonin (Nichols Institute) and estradiol (Diagnostic Products Corporation, Los Angeles) by radioimmunoassay, and 1,25-dihydroxyvitamin D by radioreceptor assay (Nichols Institute). Vitamin D-binding protein (DBP) was measured by radioimmunoassay using standards and antiserum from Calbiochem (San Diego) (13). Intra- and interassay CVs for DBP were 6% and 10%, respectively. An index of free 1,25-dihydroxyvitamin D was calculated from the molar ratio of 1,25-dihydroxyvitamin D to DBP.

To determine the serum concentration of 25-hydroxyvitamin D, serum was extracted with acetonitrile (1:1) and the extract separated by column chromatography ( $\text{C}_{18}\text{-OH}$ ; Analytichem International, Harbor City, CA). Columns were washed with 5 mL methanol:water (70:30, by vol) and the 25-hydroxyvitamin D fraction was eluted with 5 mL hexane:dichloromethane (90:10, by vol). Concentrations of 25-hydroxyvitamin D were quantified by using normal rat serum (diluted 1:40 000 with 0.05 mol barbital acetate buffer/L containing 0.05% bovine serum albumin, pH 8.6) in a competitive protein-binding assay (14). Recovery varied from 60% to 70% and intra- and interassay CVs for 25-hydroxyvitamin D were  $\approx 10\%$ .

#### Statistical analyses

Statistical analyses were performed by using SPSS (release 4.0, 1990; SPSS Inc, Chicago). Longitudinal comparisons were made with repeated-measures analysis of variance (ANOVA). Pairwise significant differences were assessed by using Tukey's Studentized range test. Because a single missing value results in the dismissal of all data for a subject for a particular variable, missing values were interpolated by using least-squares methods. A missing value for subject *X* at time point *Y* was estimated as follows: the grand mean (for all subjects at all time points) was subtracted from the sum of the group mean at time point *Y* (the time effect) and the subject *X* mean at all time points (the subject effect). The within-cell mean square degrees of freedom was decreased by 1 for each value interpolated.

The relations among dependent variables (TPA of calcium, urinary calcium excretion, and BMD of the lumbar spine or total body) and other continuous variables measured in the study were examined by repeated-measures analysis of covariance (ANCOVA). Separate repeated-measures ANCOVAs were performed for each selected covariate. Covariates included the following: maternal age, body weight, dietary protein, dietary vitamin D, dietary sodium, dietary phosphorus, dietary fiber, dietary caffeine, amount of calcium absorbed (calculated as dietary calcium multiplied by the TPA of calcium), serum calcium, serum estradiol, the calcitropic hormones, serum osteocalcin, and urinary collagen cross-links.

Statistical analyses relevant to a single time point were performed with a Mann-Whitney *U* test. The Wilcoxon signed-rank

test was used to compare subgroups of subjects at two time points. Data are reported as means  $\pm$  SD. Analyses were considered significant when  $P < 0.05$ .

## RESULTS

Subject characteristics are shown in **Table 1**. Total maternal weight gain averaged  $17 \pm 5$  kg during pregnancy and was highly variable among women (range: 8–28 kg). Only a small amount of this weight ( $0.6 \pm 4.3$  kg) was retained postmenses. Most subjects ( $n = 12$ ) reported regular participation in physical exercise (at least once per week for  $\geq 20$  min) before becoming pregnant; the remaining two subjects were more sedentary. Most subjects reported a reduction in physical activity during pregnancy compared with prepregnancy, either in terms of intensity ( $n = 6$ ) or frequency ( $n = 4$ ). At EL and postmenses, most women ( $n = 8$ ) reported returning to their prepregnancy level of physical activity. Five women began taking an oral contraceptive postpartum: two (progestin only) 2–3 wk before and two (progestin and estrogen) 2 wk after the EL measurement and one (progestin only) 4 mo before the postmenses measurement. One of the women who began taking progestin only before EL switched to a progestin- and estrogen-containing oral contraceptive 4 mo before the postmenses measurement.

All women in the study consumed diets rich in dairy products, the primary source of their dietary calcium. Daily calcium intake (excluding the supplement) averaged 1171 mg for all six time points (**Table 2**). Compared with prepregnancy, mean dietary calcium increased significantly by  $296 \pm 314$  mg/d at T3 ( $P < 0.05$ ). On average, the changes in daily vitamin D intake paralleled those observed for dietary calcium. Although the dietary intakes of other nutrients also tended to increase during pregnancy and return to near prepregnancy amounts postpartum, none of the changes in energy, protein, phosphorus, sodium, or dietary fiber intake were significant. Conversely, caffeine intake decreased significantly during pregnancy and returned to baseline amounts postpartum ( $P < 0.001$ ).

Compared with prepregnancy, the TPA of calcium increased significantly by mean changes of 57% and 72% at T2 and T3, respectively ( $P < 0.001$ ), and returned to near the prepregnancy level at EL and postmenses (**Figure 1**). A similar response occurred in all 14 subjects. Compared with at prepregnancy, urinary calcium increased significantly by 46% at T3 and decreased significantly by 56% and 34% at EL and postmenses, respectively

( $P < 0.001$ ) (**Figure 2**). The renal response was fairly similar between subjects: all subjects had increased calcium excretion at T2 or T3, all but one had decreased excretion at EL, and all but two had decreased excretion postmenses, relative to prepregnancy.

As measured by total-body DXA, compared with prepregnancy, no change occurred in BMD as a result of pregnancy or early lactation (**Table 3**). Total-body BMD was significantly less postmenses than postdelivery, and BMD of the arms and trunk were significantly less than at prepregnancy ( $P < 0.01$ ). Although the trends for total-body BMC and BMD were similar, no significant changes were observed for total-body BMC. No change in trabecular BMD of the lumbar spine as measured by quantitative computerized tomography occurred during pregnancy, but a significant 9% loss occurred at EL compared with prepregnancy ( $P < 0.001$ ). Trabecular BMD of the lumbar spine returned to the prepregnancy value postmenses. Exclusion of the three subjects for whom prepregnancy bone measurements were unavailable did not change the significance of the results. The changes in trabecular BMD of the spine were fairly similar among subjects: all subjects had lost BMD at EL compared with postdelivery but all but one subject regained BMD postmenses compared with EL.

Compared with prepregnancy values, serum osteocalcin decreased significantly by T2 and T3 and returned to near the prepregnancy concentration at EL and postmenses ( $P < 0.001$ ) (**Figure 3**). Urinary collagen cross-links, measured relative to creatinine concentration, increased progressively at each trimester of pregnancy and remained significantly elevated at EL ( $P < 0.001$ ) but not postmenses (**Figure 4**).

Fasting serum calcium decreased slightly but significantly by T2 ( $P < 0.001$ ), returning to the prepregnancy concentration postpartum (**Table 4**). Intact PTH concentrations, although considerably more variable than serum calcium and therefore not significantly changed, followed a trend similar to that for serum calcium over the course of the study. Total 1,25-dihydroxyvitamin D and DBP concentrations increased progressively during pregnancy: they were significantly higher by T2 and T3 ( $P < 0.001$ ) and returned to the prepregnancy concentration postpartum. The index of free 1,25-dihydroxyvitamin D followed the same trend as total 1,25-dihydroxyvitamin D but was not significantly elevated above the prepregnancy concentration until T3 ( $P < 0.01$ ). No significant changes were observed in serum concentrations of 25-hydroxyvitamin D or calcitonin. Serum estradiol increased progressively and dramatically during pregnancy ( $P < 0.001$ ) and returned to near the prepregnancy concentration postpartum.

At EL, all subjects were lactating, most ( $n = 10$ ) exclusively so. Among the partial breast-feeders, two breast-fed  $\approx 80\%$  and two breast-fed  $\approx 25\%$  of total milk volume. The average breast-milk calcium concentration was  $6.96 \pm 0.82$  mmol/L (range: 5.02–8.03 mmol/L). Milk output ( $859 \pm 193$  g/d) varied considerably even among women who breast-fed exclusively (range: 596–1129 g/d). The estimated total daily secretion of calcium averaged  $6.06 \pm 1.35$  mmol/d ( $243 \pm 54$  mg/d) for women who breast-fed for 80%–100% of feedings and  $5.36 \pm 2.15$  mmol/d ( $215 \pm 86$  mg/d) when all 14 subjects were included (range: 0.97–8.13 mmol/d). Six subjects continued to breast-feed postmenses ( $3 \pm 2$  times/d; range: 1–7 times/d).

Repeated-measures ANCOVA showed that changes over time in the main outcome variables of the study (TPA of calcium, urinary calcium, and lumbar and total-body BMD) remained significant after individually controlling for other relevant variables

**TABLE 1**  
Subject characteristics<sup>1</sup>

Age (y)	29.4 $\pm$ 2.3 (25–34)
Height (cm)	164.9 $\pm$ 7.8 (150–178)
Weight (kg)	60.6 $\pm$ 7.0 (50.2–72.8)
Body mass index (kg/m <sup>2</sup> )	22.4 $\pm$ 2.7 (16.8–26.9)
Length of gestation (wk) <sup>2</sup>	39.6 $\pm$ 1.3 (37–42)
Infant birth weight (kg)	3.7 $\pm$ 0.4 (2.8–4.2)
Duration of lactation (mo)	12 $\pm$ 10 (2–34)
Duration of postpartum amenorrhea (mo)	8 $\pm$ 3 (3–14)

<sup>1</sup>  $\bar{x} \pm$  SD; range in parentheses;  $n = 14$ . Prepregnancy data were reported for age, height, weight, and body mass index for all subjects, except first trimester data were used for age, height, and weight for one subject and for height for another subject for whom prepregnancy data were not available.

<sup>2</sup> Determined as the time between the first day of the last menstrual period before conception and the infant's birth.

TABLE 2

Dietary intakes estimated from 3-d weighed food records, by study time point<sup>1</sup>

	Prepregnancy (n = 13) <sup>2</sup>	T1 (n = 14)	T2 (n = 14)	T3 (n = 14)	EL (n = 14)	Postmenses (n = 14)	P <sup>3</sup>
Energy (kJ/d)	8142 ± 1749	8996 ± 2159	8678 ± 2000	9192 ± 2389	8406 ± 1816	8289 ± 2067	NS
Protein (g/d)	85 ± 16	87 ± 18	93 ± 18	92 ± 18	94 ± 20	78 ± 20	NS
Calcium (mg/d) <sup>4</sup>	1054 ± 262 <sup>a</sup>	1239 ± 282 <sup>a,b</sup>	1202 ± 327 <sup>a,b</sup>	1350 ± 319 <sup>b</sup>	1119 ± 313 <sup>a,b</sup>	1055 ± 396 <sup>a</sup>	< 0.05
Phosphorus (mg/d) <sup>4</sup>	1486 ± 330	1513 ± 347	1570 ± 345	1640 ± 332	1545 ± 250	1398 ± 506	NS
Vitamin D (µg/d) <sup>4</sup>	5.4 ± 2.7 <sup>a,b</sup>	6.2 ± 2.7 <sup>a,b</sup>	6.3 ± 2.4 <sup>a,b</sup>	7.0 ± 3.2 <sup>b</sup>	5.4 ± 2.4 <sup>a,b</sup>	4.4 ± 2.9 <sup>a</sup>	< 0.05
Sodium (mg/d)	2908 ± 885	3108 ± 1041	2719 ± 913	3035 ± 561	2914 ± 533	2887 ± 977	NS
Dietary fiber (g/d)	20 ± 6	18 ± 7	20 ± 6	22 ± 8	17 ± 5	17 ± 8	NS
Caffeine (mg/d)	74 ± 76 <sup>a,b</sup>	19 ± 25 <sup>a</sup>	13 ± 26 <sup>a</sup>	18 ± 35 <sup>a</sup>	59 ± 84 <sup>a,b</sup>	80 ± 97 <sup>b</sup>	< 0.001

<sup>1</sup>  $\bar{x} \pm SD$ . Means with different superscript letters are significantly different,  $P < 0.05$  (Tukey's test). T1, T2, and T3, first, second, and third trimesters of pregnancy; EL, early lactation.

<sup>2</sup> Missing values were interpolated by using least-squares methods.

<sup>3</sup> Differences across groups, by repeated-measures ANOVA.

<sup>4</sup> Does not include daily supplement containing 160 mg Ca, 125 mg P, and 10 µg vitamin D.

included in the study. Total serum 1,25-dihydroxyvitamin D, but not the index of free 1,25-dihydroxyvitamin D, was positively associated with the TPA of calcium ( $P < 0.05$ ). Dietary sodium ( $P < 0.05$ ), serum DBP ( $P < 0.05$ ), the index of free 1,25-dihydroxyvitamin D ( $P < 0.05$ ), serum estradiol ( $P < 0.001$ ), and the amount of calcium absorbed ( $P < 0.05$ ) were positively associated with urinary calcium. None of the concurrently measured factors examined were associated with lumbar BMD. There was a positive association ( $P < 0.01$ ) between the urinary concentration of collagen cross-links and total-body BMD over the course of the study.

When compared by the Mann-Whitney  $U$  test, women who breast-fed for 80–100% of feedings at EL did not differ from other partial breast-feeders in any calcium homeostatic response. Women who continued to breast-feed postmenses did not differ with respect to recovery of lumbar or total-body BMD compared with non-breast-feeding women. Also, women who used an oral contraceptive at EL or postmenses did not differ in any calcium homeostatic response from women who did not use an oral contraceptive. When only exclusive breast-feeders and nonusers of oral contraceptives ( $n = 8$ ) were analyzed with the Wilcoxon signed-rank test, urinary calcium excretion remained significantly lower ( $P < 0.05$ ) at EL ( $2.38 \pm 1.12$  mmol/d) than at prepregnancy ( $4.66 \pm 2.24$  mmol/d). When only nonusers of oral contraceptives ( $n = 9$ ) were analyzed with the Wilcoxon signed-rank test, urinary calcium excretion remained significantly lower ( $P < 0.05$ ) postmenses ( $3.11 \pm 1.62$  mmol/d) than at prepregnancy ( $3.90 \pm 2.44$  mmol/d).

## DISCUSSION

Many cross-sectional and short-term longitudinal studies of calcium homeostasis during pregnancy and lactation have been conducted. However, this is the first long-term, comprehensive, longitudinal analysis of women beginning before pregnancy and continuing postpartum (the average time between a subject's initial and final measurements was  $> 2$  y), providing a unique opportunity to detect relatively small but biologically important differences in homeostatic response over time. The homeostatic mechanisms that operate to provide maternal calcium differed during pregnancy, lactation, and after the resumption of menses.

## Intestinal calcium absorption

Calcium absorption increased during pregnancy but did not differ at EL compared with that at prepregnancy, consistent with previous studies (15–19). Although the rise in calcium absorption from 32.9% at prepregnancy to 36.0% postmenses was not significant [given a type I error of 0.05, a power of 90%, and a within-subject SD of 6.3 (estimated from data); a minimum difference in the TPA of calcium of 7.8% was detectable in the present study], the direction of the change was consistent with the significant increase from 31% to 37% reported in a cross-sectional study of women in whom calcium absorption was measured 3 mo after weaning (17).

It is unlikely that concurrent changes in the intake of calcium, vitamin D, or dietary fiber substantially affected measurements of the TPA of calcium; statistically controlling for these dietary

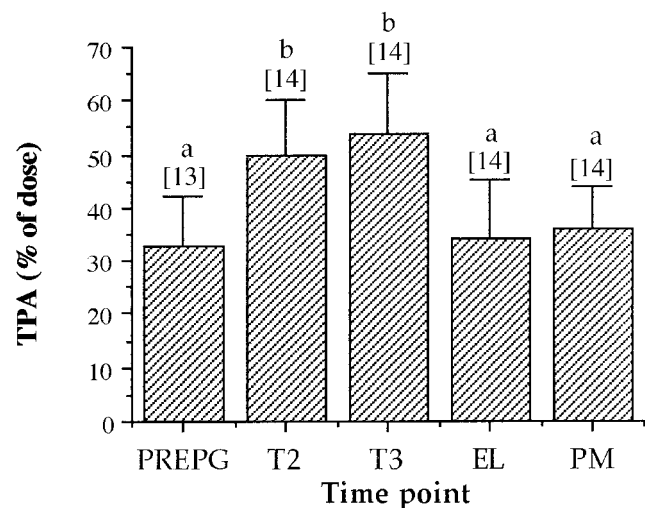
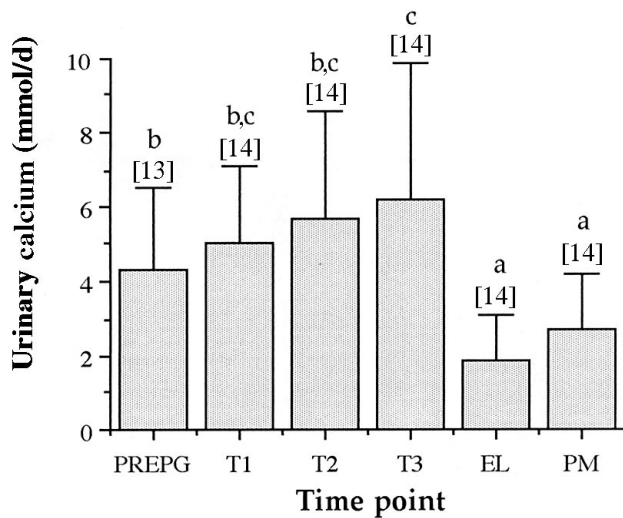


FIGURE 1. Mean ( $\pm$  SD) true percentage absorption (TPA) of calcium as determined by the 24-h urine ratio method, by study time point. PREPG, prepregnancy; T1, T2, and T3, first, second, and third trimesters of pregnancy; EL, early lactation; PM, postmenses.  $n$  in brackets; for  $n = 13$ , missing values were interpolated by using least-squares methods. Statistical significance was determined by repeated-measures ANOVA. Means with different superscript letters are significantly different,  $P < 0.001$ .



**FIGURE 2.** Mean ( $\pm$  SD) daily urinary calcium, by study time point. PREPG, prepregnancy; T1, T2, and T3, first, second, and third trimesters of pregnancy; EL, early lactation; PM, postmenses.  $n$  in brackets; for  $n = 13$ , missing values were interpolated by using least-squares methods. Statistical significance was determined by repeated-measures ANOVA. Means with different superscript letters are significantly different,  $P < 0.001$ .

factors did not alter the significance of the intestinal response over the course of the study. Rather, it is presumed that one or more hormonal mediators are involved in regulating intestinal calcium absorption. Despite slightly reduced circulating PTH concentrations, total 1,25-dihydroxyvitamin D concentrations increased progressively during pregnancy, more than doubling by T3, consistent with previous reports (16, 20–22). Additionally, 1,25-dihydroxyvitamin D concentrations were positively associated with the TPA of calcium over the course of the study. Because of corresponding changes in DBP, the index of free 1,25-dihydroxyvitamin D, the form of vitamin D presumed responsible for altering calcium absorption, did not increase significantly in this and other studies (22, 23) until T3. Although changes in the TPA of calcium have been clearly linked to 1,25-dihydroxyvitamin D in animal models, research with vitamin D-deficient rats suggests that factors independent of vitamin D

play a role in elevating calcium absorption during pregnancy (24). Other hormones that may contribute to the regulation of calcium absorption are prolactin, placental lactogen, and PTH-related protein.

Consistent with the findings of previous studies of well-nourished adult women breast-feeding a single infant (16, 25–28), none of the calcitropic hormone concentrations in the present study were significantly different between EL and prepregnancy. However, elevated concentrations of calcitropic hormones were reported in women breast-feeding twins (29) and in adolescents with low calcium intakes (27). PTH concentrations were slightly but not significantly lower postmenses than at prepregnancy. It is difficult to predict the effect, if any, of a decline in PTH on calcium absorption because 1,25-dihydroxyvitamin D concentrations were the same as before pregnancy. Cross et al (16) and Kent et al (28), in longitudinal studies, found elevated intact PTH but not 1,25-dihydroxyvitamin D postweaning compared with lactation, whereas Kalkwarf et al (17), in a cross-sectional study, found elevated 1,25-dihydroxyvitamin D but not intact PTH postweaning compared with postpartum in nonlactating control subjects. The reason for this discrepancy is unclear. In the present study, earlier hormonal changes may have occurred that were not detectable by the time postmenses measurements were made at  $13 \pm 3$  mo postpartum. It is also possible that our ability to detect changes in calcitropic hormone concentrations was hampered by the fact that postmenses measurements were performed under heterogeneous conditions (eg, oral contraceptive use and continuation of breast-feeding by some subjects).

#### Urinary calcium excretion

The increase in urinary calcium excretion during pregnancy and the decline during lactation and after the resumption of menses are consistent with previous reports (16, 19, 20, 28). In the present study, the small concurrent variations in dietary calcium, sodium, protein, and caffeine were unrelated to changes in urinary calcium, as assessed by repeated-measures ANCOVA. Instead, the seemingly paradoxical wasting of calcium and other minerals (30, 31) during pregnancy may reflect the  $\approx 50\%$  elevation in glomerular filtration rate, exceeding the reabsorptive capacity of the kidney (32). Hormones may also contribute to the renal response. Although no changes in fasting calcitonin concentrations were observed in the present study, others reported

**TABLE 3**

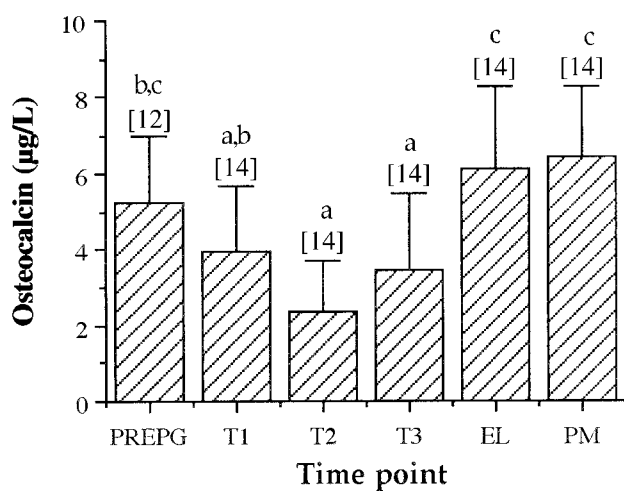
Bone mineral density (BMD) of total body and select component regions as measured by dual-energy X-ray absorptiometry, and trabecular bone mineral density of the lumbar vertebrae (L1-L2) as measured by quantitative computerized tomography<sup>1</sup>

	Prepregnancy	Postdelivery <sup>2</sup>	EL	Postmenses	$P^3$
<b>Integral BMD (g/cm<sup>2</sup>)</b>					
Arms	0.886 $\pm$ 0.055 <sup>b</sup> [10]	0.890 $\pm$ 0.051 <sup>b</sup> [13]	0.886 $\pm$ 0.048 <sup>b</sup> [13]	0.862 $\pm$ 0.047 <sup>a</sup> [13]	< 0.01
Legs	1.204 $\pm$ 0.071 [10]	1.211 $\pm$ 0.075 [13]	1.199 $\pm$ 0.074 [13]	1.198 $\pm$ 0.090 [13]	NS
Trunk	0.941 $\pm$ 0.064 <sup>b</sup> [10]	0.935 $\pm$ 0.063 <sup>a,b</sup> [13]	0.922 $\pm$ 0.067 <sup>a,b</sup> [13]	0.921 $\pm$ 0.062 <sup>a</sup> [13]	< 0.01
Total body	1.156 $\pm$ 0.055 <sup>a,b</sup> [10]	1.162 $\pm$ 0.055 <sup>b</sup> [13]	1.153 $\pm$ 0.052 <sup>a,b</sup> [13]	1.143 $\pm$ 0.050 <sup>a</sup> [13]	< 0.01
<b>Integral BMC (g)</b>					
Total body	2450 $\pm$ 294 [10]	2455 $\pm$ 279 [13]	2446 $\pm$ 287 [13]	2436 $\pm$ 258 [13]	NS
<b>Trabecular BMD (mg/cm<sup>3</sup>)</b>					
Lumbar spine	162.9 $\pm$ 25.0 <sup>b</sup> [11]	163.7 $\pm$ 25.1 <sup>b</sup> [14]	147.7 $\pm$ 21.2 <sup>a</sup> [14]	164.3 $\pm$ 21.0 <sup>b</sup> [14]	< 0.001

<sup>1</sup>  $\bar{x} \pm$  SD;  $n$  in brackets. For  $n$  values < 13, missing values were interpolated by using least-squares methods. Means with different superscript letters are significantly different,  $P < 0.05$  (Tukey's test).

<sup>2</sup> 1–2 wk postdelivery.

<sup>3</sup> Differences across groups, by repeated-measures ANOVA.



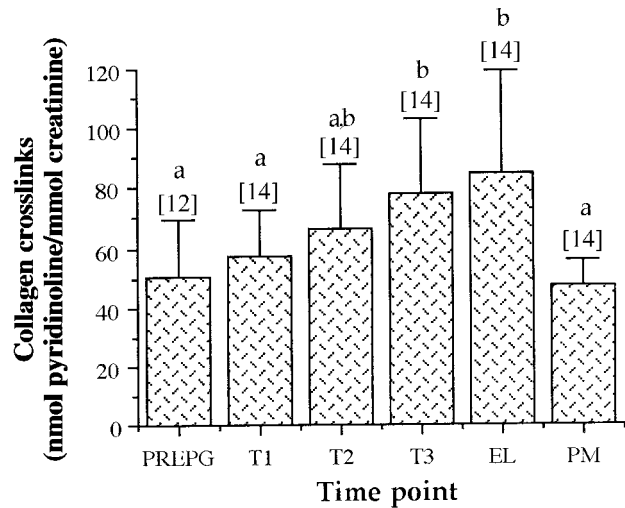
**FIGURE 3.** Mean ( $\pm$  SD) serum osteocalcin concentrations, by study time point. PREPG, prepregnancy; T1, T2, and T3, first, second, and third trimesters of pregnancy; EL, early lactation; PM, postmenses. *n* in brackets; for *n* = 12, missing values were interpolated by using least-squares methods. Statistical significance was determined by repeated-measures ANOVA. Means with different superscript letters are significantly different,  $P < 0.001$ .

that calcitonin measured at midday (at which time there is a diurnal peak) increases during pregnancy (33, 34). The extent to which small changes in midday calcitonin play a role in the overall regulation of calcium homeostasis is unclear, but an elevation is consistent with increased urinary calcium loss.

Factors responsible for the postpartum reduction in urinary calcium excretion are less clear. Neither a generalized postpartum renal conservation of minerals [eg, urinary zinc measured in the same women did not differ from values at prepregnancy (30)] nor changes in diet or calcitropic hormone concentrations are obvious explanations. The fact that the reduction in urinary calcium after parturition has been observed whether women are partially, exclusively, or not breast-feeding (19) suggests that the hormonal milieu unique to the early postpartum period, irrespective of the hormones involved in lactation, contributes to the renal response. Even after excluding partial breast-feeders ( $n = 4$ ) and oral contraceptive users ( $n = 2$ ) from the analysis, urinary calcium excretion remained significantly reduced at EL compared with prepregnancy. The postpartum decline in estradiol was inconsistent with a decrease in urinary calcium; an increase in urinary calcium has been observed as a result of the postmenopausal decline in estrogen production (35). It is possible that part of the depression in urinary calcium postmenses was due to oral contraceptive use. A decrease in urinary calcium has been observed in premenopausal women treated with an oral contraceptive (36). However, when oral contraceptive users ( $n = 5$ ) were excluded from the analysis, urinary calcium excretion remained significantly reduced postmenses compared with prepregnancy.

#### Maternal bone mineral

The finding that pregnancy does not result in a significant change in the trabecular BMD of the lumbar spine was shown in one study (37), whereas significant losses were reported in another (38). The 9% spinal trabecular bone loss measured after only 2 mo of lactation, followed by recovery postmenses, was



**FIGURE 4.** Mean ( $\pm$  SD) daily urinary collagen cross-links, by study time point. PREPG, prepregnancy; T1, T2, and T3, first, second, and third trimesters of pregnancy; EL, early lactation; PM, postmenses. *n* in brackets; for *n* = 12, missing values were interpolated by using least-squares methods. Statistical significance was determined by repeated-measures ANOVA. Means with different superscript letters are significantly different,  $P < 0.001$ .

consistent with the findings of most (16, 38–42) but not all (37) previous studies. Possible explanations for discrepancies in both pregnancy and postpartum studies, in addition to small sample size, include differences in gestational weight gain, body composition, pattern of physical activity, extent of lactation, and technique and timing of bone measurements.

This is the first published study to compare changes in total-body BMD and BMC before and after pregnancy by using DXA. The demand for calcium for fetal bone mineralization does not appear to negatively affect maternal bone among well-nourished women. At 2 mo of lactation, the most dramatic BMD loss ( $\approx 2\%$ ) compared with prepregnancy was recorded for the trunk, which is composed primarily of trabecular bone. Trabecular bone, although it makes up 20% of the total skeleton, constitutes at least half of skeletal turnover because it has a larger surface area than cortical bone (43). Therefore, it is not surprising that the measure of trabecular BMD of the lumbar spine declined 9% whereas integral BMD (trabecular and cortical bone combined) of the trunk was unchanged between postdelivery and EL. Cross et al (44) also found no change in total-body BMD or BMC as a result of lactation, as measured by DXA, but Kalkwarf and Specker (39) reported a significant loss in total-body BMC as measured by DXA. The longer period of lactation (6 mo) and the larger sample size ( $n = 65$ ) in the study by Kalkwarf et al may have been responsible for the different results. After weaning, no decrease in total-body BMC (39) or BMD (44) was found compared with during lactation or postdelivery. Our finding of a loss in total-body BMD between the postdelivery and postmenses time points is new and must be confirmed by larger studies before it can be considered physiologically significant.

It is unclear whether it is the length of lactation, length of postpartum amenorrhea, or simply the time elapsed since childbirth that is important for recovery of BMD. In a longitudinal study, Sowers et al (42) found that by 12 mo postpartum, those women who weaned at 6–9 mo had regained the spine and femoral neck

**TABLE 4**  
Fasting concentrations of serum calcium and calcitropic hormones, by study time point<sup>1</sup>

	Prepregnancy	T1	T2	T3	EL	Postmenses	P <sup>2</sup>
Calcium (mmol/L)	2.30 ± 0.07 <sup>b</sup> [12]	2.27 ± 0.08 <sup>b</sup> [14]	2.12 ± 0.12 <sup>a</sup> [14]	2.14 ± 0.15 <sup>a</sup> [14]	2.35 ± 0.10 <sup>b</sup> [14]	2.34 ± 0.15 <sup>b</sup> [14]	< 0.001
PTH (pmol/L)	3.5 ± 1.5 [12]	2.4 ± 1.0 [14]	2.1 ± 1.5 [14]	2.5 ± 1.4 [13]	3.1 ± 1.9 [12]	2.4 ± 1.2 [14]	NS
Total 1,25(OH) <sub>2</sub> D (pmol/L)	100 ± 32 <sup>a</sup> [12]	138 ± 26 <sup>a,b</sup> [14]	176 ± 36 <sup>b,c</sup> [14]	212 ± 83 <sup>c</sup> [13]	95 ± 38 <sup>a</sup> [14]	98 ± 31 <sup>a</sup> [14]	< 0.001
25(OH)D (nmol/L)	63 ± 17 [12]	52 ± 19 [14]	69 ± 41 [14]	52 ± 25 [13]	52 ± 25 [14]	52 ± 19 [14]	NS
DBP (μmol/L)	81 ± 13 <sup>a</sup> [12]	104 ± 32 <sup>b,c</sup> [13]	126 ± 41 <sup>d</sup> [14]	118 ± 29 <sup>c,d</sup> [13]	83 ± 18 <sup>a,b</sup> [14]	87 ± 15 <sup>a,b</sup> [11]	< 0.001
IF 1,25(OH) <sub>2</sub> D (molar ratio)	1.2 ± 0.4 <sup>a</sup> [12]	1.5 ± 0.6 <sup>a,b</sup> [13]	1.6 ± 0.8 <sup>a,b</sup> [14]	1.9 ± 0.8 <sup>b</sup> [13]	1.2 ± 0.4 <sup>a</sup> [14]	1.2 ± 0.5 <sup>a</sup> [11]	< 0.01
Estradiol (nmol/L)	0.61 ± 0.27 <sup>a</sup> [12]	3.45 ± 1.36 <sup>a</sup> [14]	41.03 ± 9.58 <sup>b</sup> [14]	68.86 ± 13.11 <sup>c</sup> [14]	0.25 ± 0.11 <sup>a</sup> [12]	0.44 ± 0.40 <sup>a</sup> [14]	< 0.001
Calcitonin (ng/L)	12 ± 4 [12]	11 ± 4 [14]	10 ± 2 [14]	10 ± 3 [13]	11 ± 6 [13]	8 ± 3 [14]	NS

<sup>1</sup>  $\bar{x} \pm SD$ ; *n* in brackets. For *n* values < 14, missing values were interpolated by using least-squares methods. Means with different superscript letters are significantly different, *P* < 0.05 (Tukey's test). T1, T2, and T3, first, second, and third trimesters of pregnancy; EL, early lactation; PTH, parathyroid hormone; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; DBP, vitamin D-binding protein; IF 1,25(OH)<sub>2</sub>D, index of free 1,25(OH)<sub>2</sub>D expressed as a molar ratio of 1,25(OH)<sub>2</sub>D to DBP × 10<sup>6</sup>.

<sup>2</sup> Differences across groups, by repeated-measures ANOVA.

BMD lost during lactation, whereas those women who continued to breast-feed had significantly lower values than those at the postpartum baseline measurement. In our study, the final bone measurement was made on the basis of the resumption of menses, rather than on the basis of weaning or time elapsed since parturition, as was done in most previous studies. By ≈5 mo postmenses, even the women who continued to breast-feed were able to replace previously lost spinal trabecular bone. Sowers et al (45) also showed bone recovery in women during a subsequent pregnancy. The ovarian, pituitary, or calcitropic hormones responsible for the loss of bone during early lactation and subsequent replacement after menses resumes remain to be determined.

A decline in serum osteocalcin and increase in urinary cross-links during pregnancy is consistent with the findings of previous reports (16, 21, 46). However, because of hemodilution, fetal contribution, increased turnover of nonbone tissue, and an elevated glomerular filtration rate, it is difficult to interpret what these changes in biochemical markers of bone turnover signify. It is possible that bone turnover increases during pregnancy without a net change in maternal bone. The return of osteocalcin to prepregnancy concentrations during lactation was reported in a similar longitudinal study (16), but other studies of women later in lactation have found osteocalcin concentrations higher than in nonlactating control subjects (28, 47). The elevation in urinary cross-links during lactation is consistent with the findings of other studies of biochemical markers of bone resorption (16, 28, 47). Together the bone marker results suggest increased bone turnover during lactation with bone loss exceeding bone synthesis, consistent with the direct measurement of bone loss. The decline in urinary cross-links postmenses relative to those at EL is consistent among studies, but a concurrent decline in osteocalcin has also been reported (16, 28). It is unclear to what extent differences in the timing of measurements may contribute to the discrepancies between studies. The slight elevation in osteocalcin relative to collagen cross-links is consistent with bone replacement.

## Summary

This unique long-term, longitudinal analysis suggests that the homeostatic mechanisms that operate to provide calcium differ during pregnancy, during lactation, and after the resumption of menses. Despite increased urinary calcium losses, increased dietary calcium and intestinal calcium absorption appeared to provide the calcium required for fetal bone mineralization, without necessitating a substantial net loss in maternal bone mineral. During early lactation, renal calcium conservation and spinal bone mineral resorption occurred. After the resumption of menses, renal calcium conservation appeared to continue and spinal bone mineral was recovered. ☞

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