Negative calcium balance during lactation in rural Mexican women

Soledad DeSantiago, Leticia Alonso, Ali Halhali, Fernando Larrea, Fernando Isoard, and Héctor Bourges

ABSTRACT

Background: Additional calcium is required during lactation, and several calcium regulatory factors are involved in calcium balance. In lactating rural women who have marginal nutrition and consume a high-fiber diet, negative calcium balance may be expected.

Objective: We evaluated calcium balance and its association with potential calcium regulatory factors in lactating, rural Mexican women who had marginal nutrition and consumed a high-fiber diet.

Design: This cross-sectional study included women at 1, 3, 6, and 12 mo of lactation (L1, L3, L6, and L12 groups) and women who had weaned their infants (W group). Age-matched, nonlactating women (NL group) were also included. Calcium balance and concentrations of calcium regulatory factors were determined. Correlation analysis was performed by using data from all of the lactating women.

Results: Calcium balance in the L1, L3, and L6 groups was negative and was significantly different ($P < 0.05$) from that in the W and NL groups. Serum parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25-(OH)2D] concentrations were significantly higher ($P < 0.05$) in the W group than in the L and NL groups. Calcium balance was positively associated with serum estradiol concentrations ($r = 0.58$, $P < 0.05$) and negatively associated with serum 1,25-(OH)2D concentrations ($r = -0.52$, $P < 0.05$). Breast-milk calcium concentrations correlated positively with serum PTH-related peptide (PTHrP) concentrations ($r = 0.51$, $P < 0.05$) and negatively with serum estradiol concentrations ($r = -0.57$, $P < 0.05$).

Conclusions: Negative calcium balance was observed during lactation in rural Mexican women who consumed a high-fiber diet. Furthermore, the data suggest that the hormones estradiol and PTHrP are involved in the regulation of calcium balance and of the calcium content of milk during lactation. Am J Clin Nutr 2002;76:845–51.

KEY WORDS Calcium balance, calcitrophic hormones, parathyroid hormone–related peptide, PTHrP, insulin-like growth factor I, IGF-I, lactation, rural Mexican women

INTRODUCTION

Lactation imposes a particular burden on the calcium economy of the mother because production of milk represents an additional output for this nutrient. According to an earlier report (1), negative calcium balance was observed in well-nourished nursing women. In addition, a decrease in bone mineral density in healthy nursing mothers was documented (2–8). Changes in markers of bone turnover during lactation were also reported (4, 9). Bone loss has been associated with changes in serum concentrations of parathyroid hormone–related peptide (PTHrP), prolactin, and estradiol, but not with calcitrophic hormones such as parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25-(OH)2D] (10, 11). In addition, previous studies (4, 6, 8) showed that breast-milk calcium secretion is independent of calcium intake. Taken together, these observations suggest that bone may represent the main source of this nutrient in milk.

In women from rural areas in central and southern Mexico, lactation is usually the exclusive form of infant feeding for at least the first 6 mo and often until the 12th month after birth (12). Previous studies (13–15) showed that the high fiber content of rural diets results in low calcium bioavailability. Therefore, lactation may impair calcium balance in rural women. The purpose of this study was to evaluate calcium balance and its association with potential calcium regulatory factors in lactating rural women who had marginal nutrition and consumed a high-fiber diet.

SUBJECTS AND METHODS

Subjects

The balance study was done at the metabolic facilities of a rural hospital belonging to the Nutrition Research Unit of the Mexican Institute of Social Security; the hospital is located in the Otomi Indian community of San Mateo Capulhuac in the central area of Mexico. The study protocol was approved by the Ethics Committee on Human Research of the Mexican Institute of Social Security.

This cross-sectional study included 27 lactating women (the L groups), 7 nonpregnant, nonlactating women (the NL group), and 7 women who had delivered their infants within the past 16 mo and had weaned their infants in the past 2 mo (the W group); all of the women belonged to a community that was described previously (16). The group of lactating women included those who...
were at 1, 3, 6, and 12 mo of lactation (the L1, L3, L6, and L12 groups, respectively). The infants of all of the mothers in the L1, L3, and L6 groups were exclusively breast-fed, and the infants of the mothers in the L12 group were partially breast-fed. The women in the NL, L12, and W groups had regular menses. The resumption of menses in the L12 and W groups occurred between 31 and 39 wk postpartum. Women were included in the study if they were 18–35 y of age, had given birth 2–6 times, had a body mass index of 19–24, did not smoke, did not take oral contraceptives, did not have evidence of chronic illness or a history of alcohol abuse, and voluntarily signed an informed consent statement. Women were excluded from the study if they were primigravida, had a complicated pregnancy or a premature delivery, or had delivered an infant with a birth weight <2500 g.

**Metabolic balance protocol and dietary intake**

Subjects consumed a constant, controlled diet for 10 d. On the sixth day, they were admitted to the metabolic unit, where they remained during the last 4 d for calcium balance studies. The diet during the balance period was designed to resemble the habitual diet of each woman on the basis of a dietary survey carried out by a trained observer at the subject’s home (15, 16). The survey for each subject was conducted on 2 weekdays and a weekend day and combined weighing, recording, and recall techniques (17); nutrient intakes were calculated by using the Mexican food composition tables of the National Institute of Medical Sciences and Nutrition Salvador Zubirán (18). The diet consisted of corn tortillas, beans, tomatoes, onions, green chilies, potatoes, and bananas; egg was the source of animal protein, and sesame seed oil was the source of fat. Energy intake was 200 kJ · kg body wt⁻¹ · d⁻¹, of which 17% was from fat and 10% was from protein; carbohydrates were adjusted to maintain a constant proportion with lipids. The calcium content of the diet was 750 mg/d, and the average content of dietary fiber was 32 g/d. The diet for the balance period was divided into daily portions and kept refrigerated or frozen as appropriate; duplicate portions were reserved for composition analysis. During the calcium balance study, dietary calcium, fecal calcium excretion, 24-h urinary calcium, and breast-milk calcium were determined. In addition, the following serum concentrations were also measured: intact PTH; 1,25-(OH)₂D; prolactin; estradiol; PTHrP; insulin-like growth factor I (IGF-I); osteocalcin; N-telopeptides; calcium; and phosphorus. For the women in the NL, L12, and W groups, the study was conducted during the follicular phase of their menstrual cycle (day 7).

**Sample collection**

During the last 4 d of the balance study, preweighed diets were provided to the subjects in the metabolic unit, and the leftovers were weighed and recorded. Twenty-four-hour urine samples were collected in acid-washed plastic bottles containing 20 mL of a 6-mol HCl/L solution. Four 24-h feces samples were collected in acid-washed containers, pooled with the use of a qualitative marker (vegetable charcoal), and refrigerated immediately after defecation. Stool weight, dry matter excretion, and the number of fecal samples (laxation rate) were recorded daily during the balance period. Representative 24-h milk samples were obtained at 0800 after the women had fasted overnight. Blood samples were collected in evacuated tubes with protease inhibitors, placed on ice, and centrifuged at 1400 × g for 10 min at 4°C. Serum aliquots for biochemical analyses were stored at −70°C.

**Biochemical analyses**

The energy content of the diet was determined by using bomb calorimetry (model 1261; Parr Instruments, Moline, IL) (21), and lipid and nitrogen contents were determined as previously reported (16, 22, 23). Samples of the diet, feces, and breast milk were dry-ashed, diluted with deionized water, and analyzed for calcium content by using atomic absorption spectrophotometry (model 2380; Perkin-Elmer, Norwalk, CT) (23). Urinary calcium was measured after adjusted dilution with lanthanum chloride solution (1:100 by vol). Recovery of calcium from the diet and from feces, urine, and breast milk ranged between 96% and 100%. Intact PTH was determined by using an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) with intraassay and interassay CVs of 1.3–3.4% and 5.1–6.1%, respectively. PTHrP was also determined by using an immunoradiometric assay (Nichols Institute Diagnostics) with intraassay and interassay CVs of 5.1–10.7% and 8.7–11.2%, respectively. PTHrP was detectable in all samples at a lower limit of detection of 2.6 ng/L. Prolactin and estradiol were measured by using a specific double-antibody radioimmunoassay (24) with intraassay and interassay CVs of 3.4–6.7% and 5.4–7.3%, respectively, for prolactin and 4.5–7.8% and 5.2–8.4%, respectively, for estradiol. Osteocalcin was determined by using a radioimmunoassay (Diagnostic Systems Laboratories, Inc, Webster, TX) with intraassay and interassay CVs of 2.1–4.3% and 4.2–6.8%, respectively, and N-telopeptides were determined by using an enzyme-linked immunosorbent assay (Oxest, Seattle). IGF-I serum concentrations were measured by using a radioimmunoassay (Nichols Institute Diagnostics) with intraassay and interassay CVs of 2.4–3.0% and 5.2–8.4%, respectively, and 1,25-(OH)₂D was determined by using a radioreceptor assay (Nichols Institute Diagnostics) with intraassay and interassay CVs of 5.4–10.6% and 9.3–15.3%, respectively. Serum and urine assays for creatinine (25) were run on an Analyzer 2 (Beckman, Fullerton, CA), serum calcium was determined by using atomic absorption spectrophotometry (26), and serum phosphorus was determined by using spectrophotometry (model DU-70; Beckman) (27). Calcium excretion after correction for glomerular filtration rate was calculated as follows: urine Ca × serum creatinine/urine creatinine. All biochemical analyses were performed in batches so that all samples from the study were processed at the same time.

**Balance calculations and statistical analysis**

The calcium balance was calculated by using the following formula:

\[
\text{Calcium balance} = \text{calcium intake} - (\text{urine calcium} + \text{feces calcium} + \text{milk calcium})
\]

Statistically significant differences between groups were established by using analysis of variance with a Bonferroni test.
Spearman rank correlation analysis was performed on the data from all of the lactating women to evaluate associations between the biochemical indexes (intact PTH, 1,25-(OH)2D; prolactin; estradiol; PTHrP; IGF-I; osteocalcin; and N-telopeptide) and the variables of calcium balance (calcium intake, fecal and urinary calcium excretion, and breast-milk calcium secretion). $P < 0.05$ was considered significant. All results were analyzed with the use of STATVIEW version 4.02 (Abacus Concepts, Berkeley, CA).

**RESULTS**

**Subject characteristics and dietary intake**

Subject characteristics are shown in Table 1. None of the women were undernourished on the basis of their body mass index values, and all of the women found to be clinically healthy by medical examination. The dietary contents of energy, protein, calcium, phosphorus, and fiber consumed during the metabolic balance study (Table 2) were calculated as previously reported (15, 16, 28). For all of the groups combined, the mean intakes of calcium and phosphorus were 750 and 1100 mg/d, respectively, which were 75% and 93% adequate, respectively, according to dietary recommendations for lactating women (29, 30).

**Calcium balance**

Calcium balance in the L, NL, and W groups is shown in Table 3. Calcium balance was negative in all of the groups, and calcium balance study was negatively associated with milk calcium concentrations. A positive association was observed between breast-milk calcium concentrations and serum PTHrP concentrations. A significant ($P < 0.05$) lower in the L1, L3, and L6 groups than in the L12, W, and NL groups. Serum estradiol concentrations were significantly ($P < 0.001$) higher in the lactating women than in the NL and W groups. The highest prolactin concentration was observed in the L1 group. Serum estradiol concentrations were significantly ($P < 0.001$) lower in the L1, L3, and L6 groups than in the L12, W, and NL groups. Serum estradiol concentrations in the L12 and W groups were significantly different ($P < 0.05$) from those in the L1, L3, and L6 groups. Serum osteocalcin and N-telopeptide concentrations were significantly ($P < 0.05$) higher in the L6 and L12 groups than in the other groups. Serum concentrations of PTHrP, IGF-I, calcium, and phosphorus were not significantly different between the groups. PTHrP and IGF-I concentrations in milk were not significantly different between the L groups. The calcium content of milk was significantly ($P < 0.05$) higher in the L3 group than in the L1, L6, and L12 groups.

**Biochemical indexes in serum and milk**

Concentrations of biochemical index compounds in serum and milk in the L, NL, and W groups are shown in Table 4. PTH and 1,25-(OH)2D serum concentrations were significantly ($P < 0.05$) higher in the W group than in the other groups. Serum prolactin concentrations were significantly ($P < 0.001$) higher in the lactating women than in the NL and W groups. The highest prolactin concentration was observed in the L1 group. Serum estradiol concentrations were significantly ($P < 0.001$) lower in the L1, L3, and L6 groups than in the L12, W, and NL groups. Serum estradiol concentrations in the L12 and W groups were significantly different ($P < 0.05$) from those in the L1, L3, and L6 groups. Serum osteocalcin and N-telopeptide concentrations were significantly ($P < 0.05$) higher in the L6 and L12 groups than in the other groups. Serum concentrations of PTHrP, IGF-I, calcium, and phosphorus were not significantly different between the groups. PTHrP and IGF-I concentrations in milk were not significantly different between the L groups. The calcium content of milk was significantly ($P < 0.05$) higher in the L3 group than in the L1, L6, and L12 groups.

**Associations between calcium balance and biochemical index values**

As shown in Table 5, correlation analysis included all of the lactating women. Urinary calcium excretion was positively associated with serum estradiol concentrations, whereas breast-milk calcium concentrations were negatively associated with serum estradiol concentrations and with milk PTHrP concentrations. A positive association was observed between breast-milk calcium concentrations and serum PTHrP concentrations. Calcium balance was positively associated with serum estradiol concentrations and negatively associated with serum 1,25-(OH)2D concentrations. Calcium balance was negatively associated with milk calcium concentrations.
Calcium balance in nonlactating, lactating, and weaning women

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium intake (mmol/d)</th>
<th>Urine (mmol/d)</th>
<th>Feces (mmol/d)</th>
<th>Milk (mmol/d)</th>
<th>Calcium balance (mmol/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL (n = 7)</td>
<td>19.9 ± 2.9</td>
<td>1.5 ± 0.3</td>
<td>26.7 ± 6.6</td>
<td>−8.6 ± 4.7</td>
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<tr>
<td>L1 (n = 6)</td>
<td>19.8 ± 4.7</td>
<td>0.8 ± 0.2</td>
<td>29.2 ± 6.8</td>
<td>−13.1 ± 10.92</td>
<td></td>
</tr>
<tr>
<td>L3 (n = 10)</td>
<td>19.0 ± 4.1</td>
<td>0.8 ± 0.3</td>
<td>26.6 ± 7.1</td>
<td>−14.5 ± 6.92</td>
<td></td>
</tr>
<tr>
<td>L6 (n = 6)</td>
<td>19.0 ± 3.2</td>
<td>1.3 ± 0.5</td>
<td>29.8 ± 3.6</td>
<td>−15.5 ± 8.42</td>
<td></td>
</tr>
<tr>
<td>L12 (n = 5)</td>
<td>19.4 ± 3.3</td>
<td>1.5 ± 0.6</td>
<td>27.5 ± 7.1</td>
<td>−11.7 ± 10.6</td>
<td></td>
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<tr>
<td>W (n = 7)</td>
<td>19.6 ± 2.7</td>
<td>1.3 ± 0.3</td>
<td>26.1 ± 6.8</td>
<td>−7.8 ± 4.7</td>
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</table>

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium intake (mmol/d)</th>
<th>Urine (mmol/d)</th>
<th>Feces (mmol/d)</th>
<th>Milk (mmol/d)</th>
<th>Calcium balance (mmol/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL (n = 7)</td>
<td>33.2 ± 4.3</td>
<td>21.3 ± 5.7</td>
<td>23.7 ± 6.4</td>
<td>26.8 ± 5.9</td>
<td>37.6 ± 3.8</td>
</tr>
<tr>
<td>L1 (n = 6)</td>
<td>17.4 ± 1.0</td>
<td>23.4 ± 2.8</td>
<td>26.6 ± 2.6</td>
<td>25.0 ± 5.4</td>
<td>26.7 ± 1.6</td>
</tr>
<tr>
<td>L3 (n = 10)</td>
<td>3.3 ± 0.2</td>
<td>3.4 ± 0.4</td>
<td>3.8 ± 0.7</td>
<td>3.8 ± 0.4</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>L6 (n = 6)</td>
<td>7.3 ± 1.2</td>
<td>160.2 ± 9.6</td>
<td>52.8 ± 11.8</td>
<td>46.2 ± 7.9</td>
<td>29.2 ± 11.5</td>
</tr>
<tr>
<td>L12 (n = 5)</td>
<td>151.6 ± 16.4</td>
<td>22.8 ± 8.3</td>
<td>16.8 ± 2.9</td>
<td>24.2 ± 6.9</td>
<td>128.2 ± 14.7</td>
</tr>
<tr>
<td>W (n = 7)</td>
<td>134.6 ± 14.1</td>
<td>144.2 ± 30.4</td>
<td>118.6 ± 15.1</td>
<td>158.6 ± 30.3</td>
<td>142.5 ± 25.9</td>
</tr>
</tbody>
</table>

**Table 4**

Calcium loss

- Associations between calcitropic hormones and prolactin, estradiol, parathyroid hormone–related peptide, and insulin-like growth factor I
- Serum PTH and 1,25-(OH)2D concentrations were positively associated ($r = 0.53$, $P = 0.04$). No significant associations were found between serum concentrations of PTH or 1,25-(OH)2D and serum concentrations of prolactin, estradiol, PTHrP, or IGF-I. No correlation was observed between serum concentrations of prolactin or estradiol and serum or milk concentrations of PTHrP or IGF-I.
- To determine whether the concentrations of calcium regulatory factors were associated with the time of lactation, a correlation analysis was performed. Serum estradiol, osteocalcin, and N-telopeptide concentrations correlated positively with time of lactation ($r = 0.57$) ($P = 0.02$), $r = 0.70$ ($P = 0.004$), and $r = 0.65$ ($P = 0.01$), respectively. In contrast, serum prolactin correlated negatively with time of lactation ($r = -0.60$, $P = 0.01$). However, 1,25-(OH)2D, PTH, PTHrP, and IGF-I were not significantly associated with time of lactation.

**Discussion**

The data obtained in the present study showed a negative calcium balance during lactation in women from a rural Mexican community who consumed a high-fiber diet. In this study we used an appropriate calcium balance method, which was carefully and strictly controlled. However, one limitation of the balance technique was that nutrient intakes during the controlled period were constant, unlike the natural variability of nutrient intakes in the habitual diet. Because the study was performed over a short period, it is not appropriate to assume that the observed negative calcium balance will be maintained during a more prolonged time. In addition, this short period may explain the absence of hypocalcemia and therefore the increase in circulating PTH concentrations.
Despite this limitation, use of the balance method in the present study showed a clear calcium deficit in rural lactating women who consumed 32 g fiber/d and 750 mg Ca/d in their diet. The high fiber content and chemical composition of calcium salts in the habitual Mexican rural diet may lead to a negative calcium balance by impairing intestinal calcium absorption (13, 17). In addition to dietary fiber, other factors, such as the calcium content of digestive secretions (endogenous calcium), an insufficiently long adjustment period during the balance studies, and subjects who are “poor absorbers” (31), may be involved in fecal calcium excretion. In another study of ours (S. DeSantiago L. Alonso, F. Perea, F. Jasso, A. Halhali, unpublished observations, 2002), lactating women who had a calcium intake (750 mg/d) similar to that in the present study and who consumed a low-fiber diet (14 g/d) had lower fecal calcium excretion than did those in the present study, resulting in a higher calcium bioavailability (an increase of 200 mg/d in apparent intestinal calcium absorption). However, we do not have any calcium balance data from that study because milk and urine samples were not collected. In other calcium balance studies (32, 33) conducted during pregnancy, negative calcium balance and high fecal calcium excretion were found in >30% of the subjects during their first trimester of pregnancy. In the present study, high fecal calcium excretion was observed in all of the groups, and there were no significant differences in fecal calcium excretion between the groups. These results agree with those of a previous report showing no change in intestinal calcium absorption during lactation (34). Support for these observations comes from the present study and other studies (35, 36), in which 1,25-(OH)2D serum concentrations did not differ significantly between the NL and L groups. Calcium balance data showed that the deficit in calcium was significantly higher in all of the L groups than in the NL and W groups. The presence of this negative calcium balance condition may reflect an adaptive response to the existence of a high calcium bone turnover. It was shown previously (3–9) that calcium concentrations in the serum and milk of lactating women are maintained through decreased renal excretion of calcium and increased bone calcium resorption. In the present study, urinary calcium excretion tended to be lower in the L1 and L3 groups than in the other L groups or in the NL and W groups. Decreased urinary calcium excretion during the first 3 mo of lactation cannot be explained by changes in PTH serum concentrations but can be explained by another factor involved in the regulation of tubular renal calcium reabsorption, PTHrP, which is a hormone that is involved in calcium metabolism during lactation (10). In the present study, serum PTHrP concentrations were significantly associated with the calcium content of milk \(r = 0.51, P < 0.05\). This observation suggests that PTHrP may participate in the process of calcium transport from the circulation to the mammary glands. IGF-I is involved in the increased incorporation of proline into bone collagen during treatment with PTHrP (40). To our knowledge, there is no previous report about the association between IGF-I and PTHrP circulating concentrations during lactation. In the present study, IGF-I concentrations were not significantly different between the groups, and no correlations were observed between PTHrP and IGF-I or between PTHrP and calcium balance or lactation time.

Women who had weaned their infants and resumed menses had significantly higher serum PTH and 1,25-(OH)2D concentrations than did lactating women (41, 42). In addition, those women had a less negative calcium balance than did their amenorrheic counterparts. These results suggest that ovarian and pituitary factors during lactation may be additional factors involved in bone calcium turnover. In the present study, serum estradiol concentrations were negatively associated with serum prolactin concentrations. A high prolactin concentration during lactation inhibits pituitary gonadotropins and estradiol production by the ovary (24). Prolactin stimulates bone calcium mobilization directly (10, 11, 43) or by altering estradiol (10, 11, 44) and 1,25-(OH)2D production (45). In addition, estrogens are implicated in bone turnover, and low estradiol concentrations may cause bone resorption. In the present study, the observation that serum prolactin concentrations did not correlate with calcium balance suggests that this hormone may not be involved directly in bone calcium metabolism but rather indirectly through its effects at the level of the hypothalamic-pituitary-ovarian axis. Although the lack

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### TABLE 5

<table>
<thead>
<tr>
<th></th>
<th>Urinary calcium excretion</th>
<th>Breast-milk calcium</th>
<th>Calcium balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P^*)</td>
<td>(r)</td>
</tr>
<tr>
<td>PTH</td>
<td>0.01</td>
<td>0.98</td>
<td>0.07</td>
</tr>
<tr>
<td>1,25-(OH)2D</td>
<td>0.04</td>
<td>0.86</td>
<td>0.28</td>
</tr>
<tr>
<td>Prolactin</td>
<td>-0.41</td>
<td>0.09</td>
<td>-0.15</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.53</td>
<td>0.03</td>
<td>-0.57</td>
</tr>
<tr>
<td>Serum PTHrP</td>
<td>0.11</td>
<td>0.66</td>
<td>0.51</td>
</tr>
<tr>
<td>Serum IGF-I</td>
<td>0.05</td>
<td>0.84</td>
<td>-0.13</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>0.14</td>
<td>0.58</td>
<td>0.04</td>
</tr>
<tr>
<td>Milk PTHrP</td>
<td>0.05</td>
<td>0.85</td>
<td>-0.53</td>
</tr>
<tr>
<td>Milk IGF-I</td>
<td>0.14</td>
<td>0.55</td>
<td>0.14</td>
</tr>
<tr>
<td>Milk calcium</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Analyzed by Spearman rank correlation. \(P < 0.05\) was considered significant.
of a significant association between calcium balance and prolactin may be due to the difficulty in obtaining a representative sample for prolactin analysis, the increase in serum prolactin throughout lactation, combined with low serum estradiol concentrations, may provide a synergistic milieu that promotes bone resorption.

In summary, lactating women from a rural Mexican community who consumed a typical high-fiber diet had negative calcium balance. This condition was not associated with calcium intake or with serum concentrations of PTH, IGF-I, or prolactin. In contrast, serum estradiol concentrations were positively associated with calcium balance and negatively associated with breast-milk calcium. In addition, serum PTHrP was positively correlated with the calcium content of milk. The data support the hypothesis that the hormones estradiol and PTHrP are involved in the regulation of calcium balance and of the calcium content of milk during lactation.

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