Calcium homeostasis during pregnancy and lactation in Brazilian women with low calcium intakes: a longitudinal study\textsuperscript{1–4}

Carmiña L Vargas Zapata, Carmen M Donangelo, Leslie R Woodhouse, Steven A Abrams, E Martin Spencer, and Janet C King

ABSTRACT

Background: Physiologic adjustments in calcium homeostasis during pregnancy and lactation in women with marginal calcium intakes have not been described.

Objective: The objective was to examine longitudinal changes in various aspects of calcium homeostasis during pregnancy and lactation in 9 healthy Brazilian women who habitually consumed \textasciitilde500 mg Ca/d.

Design: Calcium homeostasis was assessed at 3 time points: 10–12 (early pregnancy, EP) and 34–36 (late pregnancy, LP) wk of pregnancy and 7–8 wk postpartum (early lactation, EL). At each time point, the following variables were measured: dietary calcium intake with a 3-d weighed food record, 24-h urinary calcium excretion (UCa), intestinal calcium absorption (%CaAbs) via administration of stable calcium isotopes with a breakfast meal, serum 1,25-dihydroxyvitamin D, parathyroid hormone (PTH), insulin-like growth factor I (IGF-I), and biochemical markers of bone turnover.

Results: Dietary calcium did not change during the study. %CaAbs increased from 69.7 \pm 5.4\% (\pm SEM) during EP to 87.6 \pm 4.5\% during LP (\textit{P} < 0.05) and returned to 65.1 \pm 6.2\% during EL. Compared with EP, UCa decreased 22\% during LP and 68\% during EL (\textit{P} < 0.05). The net mean change in calcium retention was 212 mg/d during LP and 182 mg/d during EL. Several significant associations were found between the main outcome variables (%CaAbs, UCa, and markers of bone turnover) and serum hormones, especially IGF-I and PTH.

Conclusions: Calcium homeostasis appears to be attained by a more efficient intestinal calcium absorption during pregnancy and by renal calcium conservation during both pregnancy and lactation. IGF-I and PTH seem to play major roles in the adjustment of calcium metabolism during pregnancy and lactation. \textit{Am J Clin Nutr} 2004;80:417–22.

KEY WORDS Pregnancy, lactation, calcium intake, calcium absorption, urinary calcium, calciotropic hormones, insulin-like growth factor I, Brazilian women

INTRODUCTION

During pregnancy and lactation, \textasciitilde200–300 mg Ca/d is either transferred via the placenta to the fetus or excreted in breast milk (1, 2). The provision of calcium during pregnancy and lactation requires physiologic adaptation of calcium homeostatic mechanisms, including intestinal calcium absorption, urinary calcium excretion, and maternal bone calcium turnover. Studies of calcium homeostatic responses during pregnancy and lactation have been done mainly in women with relatively high calcium intakes (>1000 mg/d) and have shown increases in both intestinal calcium absorption and urinary calcium excretion during pregnancy and increased rates of bone turnover during both pregnancy and lactation (1–3). Much less is known about the adjustments in calcium homeostasis during pregnancy and lactation in women who habitually consume diets with marginal amounts of calcium (2, 4). In these women, physiologic responses and hormonal regulation may be quantitatively and qualitatively different from those in women with higher intakes.

In a previous longitudinal study by Ritchie et al (5), the effect of pregnancy and lactation on intestinal calcium absorption, urinary calcium, mineral bone density, hormones regulating calcium metabolism, and markers of bone turnover were examined in American women who habitually consumed 1200 mg Ca/d. In the present study, most of these indexes were evaluated in Brazilian women who habitually consumed \textasciitilde500 mg Ca/d. The objective of the present study was to examine longitudinal changes in the efficiency of intestinal calcium absorption, urinary calcium excretion, hormonal regulation, and biochemical markers of bone turnover in the Brazilian women during pregnancy and lactation.

SUBJECTS AND METHODS

Subjects

The subjects were recruited at Maternidade Escola of the Federal University of Rio de Janeiro, Brazil, during their first visit for

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\textsuperscript{2} Presented in part at the FASEB meeting held in San Diego, April 14–18, 2000.

\textsuperscript{3} Supported in part by a gift from the Center for Research and Information on Nutrition, Paris. CMD is a Research Fellow of Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

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prenatal care. A full explanation of all the study procedures was given to the subjects. Nine adult women of low socioeconomic status participated in the study. The women were healthy, non-smoking, nonphysically active multigravidae and had no history of health problems or complications during previous pregnancies. All of the women were of mixed black and white ethnicity. All of the women used iron supplements during the second half of their pregnancies, but none used calcium or other vitamin-mineral supplements. Iron supplements were not used after delivery. The women maintained their habitual dietary habits during the study. The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and by the Ethical Committee of Maternidade Escola, Universidade Federal do Rio de Janeiro. Informed written consent was obtained from all subjects.

Study design

The subjects were examined at 3 time points: 10–12 (early pregnancy, EP) and 34–36 (late pregnancy, LP) wk of pregnancy and 7–8 wk postpartum (early lactation, EL). All women exclusively breastfed their infants. At each time point the following were assessed: habitual dietary calcium intake, 24-h urinary calcium excretion, efficiency of intestinal calcium absorption, and blood and urine biochemical indexes of calcium and bone metabolism.

Procedures

Dietary intake was assessed from weighed food intake records kept by the subjects for 3 consecutive days before each clinical test at the 3 time points of the study. The subjects were carefully instructed and trained by one of the investigators (CVZ) on the food-weighing and-recording procedures. Dietary nutrient intakes were determined by using THE FOOD PROCESSOR (ESHA Research, Salem, OR), which was adapted for Brazilian foods based on published food-composition data (6). Phytate intake was determined by using data from Harland and Oberleas (7).

Urine (50 mL) and blood (10 mL) samples were obtained after the subjects had fasted overnight (10 h) for baseline measurements of calcium metabolism and of bone biochemical markers in the week before the clinical test at each time point.

The clinical tests for calcium absorption were performed after an overnight fast at the Maternidade Escola, Federal University of Rio de Janeiro, by using a double-tracer isotope technique (8). The stable isotopes 45Ca and 46Ca were used as intravenous and oral tracers, respectively. Isotopes were purchased as calcium carbonate (42CaCO3, 93.58% enrichment, and 46Ca CO3, 6.1% enrichment; Trace Science International, Toronto). Appropriate solutions were prepared from each isotope by weighing the enriched calcium carbonate to the nearest 0.01 mg, dissolving with one-fold stoichiometric excess of hydrochloric acid (Optima; Fisher Scientific, Pittsburgh), diluting the solution to volume with triple deionized water, and storing at 4 °C. Solutions for intravenous administration, which were filtered and bottled into single-dose vials, were tested for sterility and pyrogenicity by the School of Pharmacy (University of California, San Francisco). The doses administered were calculated from the total calcium concentration determined by atomic absorption flame spectrometry (AAS), isotope enrichment provided by the supplier, and weight of the administered dose.

On the morning of the test, the weights and heights of the subjects were recorded, and a baseline (fasting) blood sample (10 mL) was obtained. Subjects received a standard breakfast consisting of French bread (50 g), butter (10 g), and whole milk (50 mL) mixed with coffee (50 mL), which provided a total calcium intake of 74 mg. Breakfast was followed 10–15 min later by the ingestion of 20 μg 46Ca in 50 mL water, immediately after which 2 mL 42Ca (5.0 mg) was administered intravenously over 1–2 min into the antecubital vein with a butterfly infusion set. A complete 24-h urine collection was made during the test day. Total urine weight was measured to the nearest 0.1 g.

Urine samples were acidified (final pH: 2) by adding concentrated tr ace metal-grade hydrochloride (Fisher Scientific), and aliquots were stored at −20 °C until analyzed. Blood samples were kept refrigerated (4 °C) and processed up to 2 h after being drawn. Aliquots of serum were kept at −20 °C until analyzed.

Calcium concentrations in the urine samples were measured by AAS (model AA3300; Perkin-Elmer, Boston) in samples appropriately diluted with 0.5% lanthanum chloride (Sigma, St Louis) in 0.5 mol HCl/L (Optima; Fisher Scientific). The procedures used to prepare the stable-isotope samples and to analyze and determine the percentage absorption of calcium were as described by Ritchie et al (5). Urine samples were prepared by using the calcium oxalate precipitation method (9). Calcium isotope ratios in the samples were measured by using magnetic sector thermal-ionization mass spectrometry. The ratio of each administered tracer to 45Ca was measured, and the degree to which this ratio was increased over the natural abundance ratio was calculated. Percentage absorption of calcium was calculated as the relative recovery of the oral and the intravenous tracer in the 24-h urine samples collected after isotope administration (8, 10).

Serum calcium was measured by AAS after protein precipitation with trichloroacetic acid 0.5% and appropriate dilution with 0.1% lanthanum chloride in 0.125 mol HCl/L. Other serum components were measured as follows: osteocalcin (Metra Biosystems, Inc, Mountain View, CA), bone-specific alkaline phosphatase (Metra Biosystems, Inc), and parathyroid hormone (PTH; Diagnostic Systems Laboratories, Inc, Webster, TX) by enzyme-linked immunosorbent assay and insulin-like growth factor (IGF-I) (11) and 1,25-dihydroxyvitamin D [1,25(OH)2D] (Nichols Institute Diagnostics, San Juan Capistrano, CA) by radioimmunoassay. Cross-linked N-telopeptides of type I collagen (NTx) were measured in nonacidified urine aliquots with an enzyme-linked immunosorbent assay (Osteomark, Seattle). Creatinine concentrations in urine were measured by a method that uses picric acid (12).

Statistical analyses

The statistical analyses were performed by using STATGRAPHICS (version 7; Manugistics, Cambridge, MA). Longitudinal comparisons were made by repeated-measures analysis of variance. Pairwise significant differences were assessed by using Tukey’s range test. Associations between variables were examined by simple correlation analysis. Analyses were considered significant at P < 0.05.

RESULTS

The women studied had a normal prepregnancy body mass index and a normal blood hemoglobin concentration in EP (Table 1). Pregnancy and lactation proceeded without complications for all of the women. All of the women had a normal length of


**Characteristics of the women studied**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>31 (21–34)</td>
</tr>
<tr>
<td>BMI, before pregnancy (kg/m²)</td>
<td>23 (18–27)</td>
</tr>
<tr>
<td>Parity</td>
<td>2.6 (2–5)</td>
</tr>
<tr>
<td>Blood hemoglobin, early pregnancy (g/L)</td>
<td>121 (103–137)</td>
</tr>
<tr>
<td>Length of gestation (wk)</td>
<td>39 (37–41)</td>
</tr>
<tr>
<td>Weight gain during gestation (kg)</td>
<td>11.4 (6.5–18.9)</td>
</tr>
<tr>
<td>Infant birth weight (kg)</td>
<td>3.2 (2.7–4.4)</td>
</tr>
<tr>
<td>Infant birth length (cm)</td>
<td>51 (49–54)</td>
</tr>
</tbody>
</table>

*All values are means; range in parentheses. n = 9.*

gestational weight gain (range: 6.5–18.9 kg). Also, all of the women gave birth to healthy infants, who had an adequate birth weight (≥2.5 kg) and a normal growth rate during the lactation period studied.

All women in the study habitually consumed diets low in calcium (Table 2). The dietary calcium intake averaged 470 mg/d over the entire period of study (range: 357–746 mg/d). Dairy products contributed ≈47% of the total calcium intake. Calcium intake increased 76 mg/d (NS) during pregnancy. Dietary intakes of phosphorous, vitamin D, fiber, and phytate did not change significantly during the study.

**Dietary intakes from 3-d weighed food records during pregnancy and lactation**

<table>
<thead>
<tr>
<th>Subject</th>
<th>EP</th>
<th>LP</th>
<th>EL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1809 ± 572</td>
<td>2125 ± 373</td>
<td>2087 ± 508</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>65 ± 27</td>
<td>81 ± 26</td>
<td>65 ± 12</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>438 ± 155</td>
<td>514 ± 221</td>
<td>451 ± 180</td>
</tr>
<tr>
<td>Phosphorous (mg/d)</td>
<td>845 ± 267</td>
<td>1019 ± 255</td>
<td>884 ± 135</td>
</tr>
<tr>
<td>Vitamin D (µg/d)</td>
<td>1.9 ± 1.0</td>
<td>2.6 ± 1.6</td>
<td>2.1 ± 1.4</td>
</tr>
<tr>
<td>Phytate (mg/d)</td>
<td>1207 ± 640</td>
<td>1677 ± 842</td>
<td>1745 ± 982</td>
</tr>
<tr>
<td>Dietary fiber (g/d)</td>
<td>21.4 ± 5.9</td>
<td>21.5 ± 7.0</td>
<td>20.2 ± 8.1</td>
</tr>
</tbody>
</table>

*All values are *x* ± SD; n = 9. EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk).*

The efficiency of intestinal calcium absorption from the breakfast meal was high in all of the women throughout the study (Table 3). Compared with EP, intestinal calcium absorption increased during LP (mean increase of 26%; *P < 0.05*) and returned to the EP level at EL. The opposite was seen with the urinary excretion of calcium during pregnancy. Urinary calcium excretion decreased both during LP and EL compared with EP, with mean decreases of 22% and 68%, respectively (*P < 0.05*). Percentage calcium absorption and urinary calcium excretion were significantly associated during EP (*r* = 0.77, *P < 0.05) but not during LP or EL. The intestinal and renal responses were fairly similar in all of the women studied. All women had an increased efficiency of intestinal calcium absorption during pregnancy, and all except 2 women had decreased urinary calcium excretion in the same period. Urinary calcium excretion was lower during EL than during EP in all women.

Net change in calcium retention was estimated as the sum of mean changes in dietary calcium absorbed and urinary calcium conservation during LP and EL compared with EP, assuming that endogenous fecal calcium excretion did not change during pregnancy and lactation as was previously shown (13). The efficiency of intestinal calcium absorption from the breakfast test meal was used as a proxy to estimate calcium absorbed from the whole diet. Net change in calcium retention was mainly due to the increase in total calcium absorbed, whereas in EL it was due to the increase in urinary calcium conservation.

Fasting total serum calcium tended to decline by LP and returned to the EP concentration during EL (Table 5). Values were significantly lower during LP than during EP (*P < 0.02*). Serum PTH concentrations tended to increase during pregnancy and continued to increase during lactation; concentrations were significantly higher during EL than during EP (*P < 0.02*). Compared with EP, serum 1,25(OH)₂D increased significantly by LP (*P < 0.01*) but decreased by EL (*P < 0.01*). Serum IGF-I was also higher during LP than during EP (*P < 0.01*), but returned to EP concentrations by EL.

Biochemical markers of bone formation (serum osteocalcin and bone alkaline phosphatase) and of bone degradation (urinary NTx) increased from pregnancy to lactation (Table 5). Compared
TABLE 4
Estimated net change in dietary calcium absorption, renal calcium conservation, and calcium retention during pregnancy and lactation

<table>
<thead>
<tr>
<th></th>
<th>EP</th>
<th>LP</th>
<th>EL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary calcium absorption (mg/d)</td>
<td>$295 \pm 90^a$</td>
<td>$448 \pm 57^b$</td>
<td>$290 \pm 43^a$</td>
</tr>
<tr>
<td>Net change in dietary calcium absorption (mg/d)</td>
<td>—</td>
<td>$152 \pm 51^b$</td>
<td>$6 \pm 42^a$</td>
</tr>
<tr>
<td>Net change in urinary calcium conservation (mg/d)</td>
<td>—</td>
<td>$60 \pm 18^a$</td>
<td>$187 \pm 32^b$</td>
</tr>
<tr>
<td>Net change in calcium retention (mg/d)</td>
<td>—</td>
<td>$212 \pm 52$</td>
<td>$182 \pm 65$</td>
</tr>
</tbody>
</table>

$^a$ All values are $\bar{x} \pm SE; n = 9$. EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk). Means in a row with different superscript letters are significantly different, $P < 0.05$.

$^b$ Estimated as the product of fractional calcium absorption and daily dietary calcium intake during each time period.

$^c$ Estimated as the difference between dietary calcium absorption during LP or EL and during EP.

$^d$ Estimated as the difference between urinary calcium excretion during EP and during LP or EL.

$^e$ Estimated as the sum of net changes in dietary calcium absorption and urinary calcium conservation during LP or EL.

$^f$ Estimated as the sum of net changes in dietary calcium absorption and urinary calcium conservation during LP or EL.

with EP, serum osteocalcin and serum bone alkaline phosphatase did not change significantly by LP but increased 168% and 54%, respectively, by EL ($P < 0.01$). Serum osteocalcin concentrations were $\approx$2-fold higher during EL than during pregnancy (EP and LP) ($P < 0.01$). Urinary NTx increased significantly during pregnancy, with a mean increase of 136% from EP to LP ($P < 0.001$), and remained high during lactation. Urinary NTx concentrations were not significantly different between LP and EL.

Significant correlations were observed during pregnancy and lactation between the main outcome variables in the study: calcium absorption, urinary calcium excretion, biochemical markers of bone turnover, and serum hormone concentrations (Table 6). Percentage calcium absorption was positively associated with serum 1,25(OH)₂D and serum PTH and negatively associated with serum IGF-I during EL. Markers of bone turnover (urinary NTx and serum bone alkaline phosphatase) were associated with serum IGF-I during EP and LP; serum osteocalcin was associated with serum IGF-I during EL. Changes in calcium absorption, urinary calcium, and markers of bone metabolism were also evaluated. The increase in urinary NTx from EP to LP was associated with a corresponding increase in serum PTH ($r = 0.77$, $P < 0.05$) during LP. The decrease in urinary calcium relative to EP was positively associated with the corresponding increase in serum PTH during LP ($r = 0.79$, $P < 0.05$) and during EL ($r = 0.70$, $P < 0.05$).

DISCUSSION

The physiologic adaptation of calcium homeostasis to pregnancy and lactation in women who habitually consume marginal calcium intakes is poorly known. This is the first report of intestinal calcium absorption during pregnancy and lactation in women with low calcium intakes. The study provides evidence suggesting that the calcium needs of these women during pregnancy and lactation are met partly by the high efficiency of calcium absorption and by renal calcium conservation. Evidence is also provided that supports the hypothesis that IGF-1 and the classic calcitropic hormones PTH and 1,25(OH)₂D may play important regulatory roles in calcium homeostasis during pregnancy and lactation.

Our data indicate that intestinal and renal responses during pregnancy and lactation in women with calcium intake of $\approx$500 mg/d are qualitatively, and possibly quantitatively, different from those in women with calcium intake of $\approx$1200 mg/d (5). In the latter study, the efficiency of intestinal calcium absorption from a light breakfast meal providing $\approx$120 mg Ca (including the oral calcium isotope; LD Ritchie, personal communication, 2003) almost doubled during pregnancy (increasing from 33% to 54%) and returned to prepregnancy values during EL (34%). Urinary calcium excretion increased $\approx$17% during pregnancy but decreased to nearly 50% of the prepregnancy value during EL. These results are consistent with those of previous studies of women who were consuming calcium-rich diets (14, 15). In the present study, the efficiency of calcium absorption from a light breakfast meal providing 74 mg Ca was already high during EP (70%), increased during pregnancy from 70% to 88%, and returned to EP values during EL (65%). The small calcium load of the test meal in our study possibly contributed to the high percentage of calcium absorption at all time points (16). In contrast with the study by Ritchie et al (5), urinary calcium excretion decreased by $\approx$20% during pregnancy and by $\approx$68% during lactation. Therefore, it appears that calcium homeostasis in the women in the current study, who were accustomed to a low-calcium diet, were better able to maintain calcium homeostasis during pregnancy and lactation.

TABLE 5
Serum calcium, hormones, and markers of bone turnover during pregnancy and lactation

<table>
<thead>
<tr>
<th></th>
<th>EP</th>
<th>LP</th>
<th>EL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>$1.95 \pm 0.08^a, b$</td>
<td>$1.90 \pm 0.07^a$</td>
<td>$2.00 \pm 0.09^b$</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>$1.14 \pm 0.55^a$</td>
<td>$1.31 \pm 0.56^a, b$</td>
<td>$1.80 \pm 0.58^b$</td>
</tr>
<tr>
<td>Serum 1,25(OH)₂D (pg/mL)</td>
<td>$73.8 \pm 5.1^b$</td>
<td>$93.8 \pm 6.7^b$</td>
<td>$54.6 \pm 6.3^a$</td>
</tr>
<tr>
<td>Serum IGF-I (ng/mL)</td>
<td>$206 \pm 50^a$</td>
<td>$278 \pm 55^a$</td>
<td>$175 \pm 53^a$</td>
</tr>
<tr>
<td>Serum osteocalcin (ng/mL)</td>
<td>$1.88 \pm 0.14^a$</td>
<td>$1.83 \pm 0.14^a$</td>
<td>$5.04 \pm 3.66^b$</td>
</tr>
<tr>
<td>Serum bone alkaline phosphatase (U/L)</td>
<td>$14.1 \pm 5.1^a$</td>
<td>$19.3 \pm 5.3^b$</td>
<td>$21.7 \pm 7.8^b$</td>
</tr>
<tr>
<td>Urinary NTx (mmol/mmol creatinine)</td>
<td>$43.7 \pm 7.2^a$</td>
<td>$103 \pm 37^a$</td>
<td>$90.7 \pm 36^b$</td>
</tr>
</tbody>
</table>

$^a$ All values are $\bar{x} \pm SD; n = 9$. PTH, parathyroid hormone; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; IGF-I, insulin-like growth factor I; NTx, N-telopeptides of type I collagen; EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk). Means in a row with different superscript letters are significantly different, $P < 0.02$. 

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diet, was attained in part by an increased efficiency of intestinal calcium absorption during pregnancy and by renal calcium conservation during both pregnancy and lactation.

It is noteworthy that, at all time points during pregnancy and lactation, the efficiency of calcium absorption in the women in the current study was higher (almost doubled during EP and during EL) than that observed by Ritchie et al (5). This finding cannot be explained by the small difference in the total calcium load of the test meal used to measure calcium absorption in these studies. For instance, if the predictive equation of Heaney et al (16) were used, which calculates fractional calcium absorption as a function of the test calcium load, calcium absorption would be 42.6% before pregnancy in the study by Ritchie et al (5) and it would be 47.4% during EP in the present study. Therefore, other factors are possibly involved in the different results of these studies, such as environmental and genetic differences and the different habitual dietary calcium intakes of the women in the 2 studies.

Urinary calcium excretion in the women in the current study was high in the first trimester of pregnancy, as was observed in other studies (14, 17), probably because of the increased glomerular filtration rate that occurs by EP (3). In the current study, this high urinary calcium excretion in EP appears to reflect also the high efficiency of intestinal calcium absorption at that period, as suggested by the significant association between these 2 measurements.

The estimated net increase in calcium retention in the women in the current study during LP (212 mg/d) and EL (182 mg/d) compared with EP were close to the average estimated calcium needs for maternal-fetal transfer in the third trimester of pregnancy (200 mg/d) and for maternal-milk transfer during exclusive breastfeeding (200 mg/d) (2). Although the calcium concentration of breast milk appears to be independent of the maternal calcium intake, there is some variability in the total amount of calcium secreted daily into breast milk, usually up to 300 mg/d (2). Therefore, the physiologic intestinal and renal adaptation in the women in the current study appeared to contribute most, if not all, the calcium necessary for fetal growth and most of the calcium needed for breast-milk production.

The results of the current study are consistent with those of studies conducted in lactating women in The Gambia who were accustomed to very low calcium intakes. A high efficiency of calcium absorption was found in the Gambian women, who consumed a low-calcium diet (283 mg/d), compared with lactating women in the United Kingdom, who consumed a high-calcium diet (1168 mg/d) (18). At 3 mo postpartum, calcium absorption of isotopically enriched milk was 52% in the Gambian women and 32% in the UK women. Moreover, urinary calcium excretion was found to be much lower in the lactating Gambian women than in the lactating UK women (19).

The hormonal regulation of intestinal calcium absorption in human pregnancy and lactation has not yet been clearly identified. The active form of vitamin D probably has a role independent of calcium intake, at least during lactation, because the efficiency of calcium absorption in the lactation period was positively associated with total serum 1,25(OH)2 D in both the current study and in previous studies of women with higher calcium intakes (5, 14). Although serum 1,25(OH)2 D and serum PTH were not significantly associated in the current study, PTH probably mediates the vitamin D stimulation of intestinal calcium absorption during lactation, as suggested by the significant association that we observed between serum PTH and percentage calcium absorption in this period. The negative association that we observed between calcium absorption and serum IGF-I during EL probably reflects the stimulatory effect of IGF-I on bone turnover (20) (see below) and suggests compensatory adjustments in intestinal calcium absorption and bone turnover for maintaining calcium homeostasis during lactation.

The hormonal regulation of urinary calcium excretion during pregnancy and lactation is also not completely understood. In the current study, urinary calcium conservation in LP and EL was associated with the corresponding increase in serum PTH. PTH-induced renal calcium conservation was also observed in pregnant Malay women (21) and in pregnant and lactating adolescent Brazilian women (22). Higher serum PTH and lower urinary calcium excretion were observed in lactating Gambian women than in lactating British women (23). However, in women with habitually high calcium intakes, the association between reduced urinary calcium and increased PTH was only evident during the postweaning period or after the resumption of menses in the postpartum period (5, 14, 24). Therefore, PTH appears to play a major role in renal conservation of calcium during pregnancy and lactation, when maternal calcium intakes are low.

Markers of bone turnover appear to increase during pregnancy and lactation in women with high (>1000 mg/d) (5, 14, 25) or low (<800 mg/d) (22, 23, 25, 26) calcium intakes. Therefore, bone calcium may contribute to calcium homeostasis in pregnancy and lactation independent of maternal dietary intake.

### Table 6

<table>
<thead>
<tr>
<th>Related variables</th>
<th>EP</th>
<th>LP</th>
<th>EL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage calcium absorption and serum 1,25(OH)2 D</td>
<td>—</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Percentage calcium absorption and serum PTH</td>
<td>—</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Percentage calcium absorption and serum IGF-I</td>
<td>—</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Urinary NTx and serum IGF-I</td>
<td>0.47</td>
<td>&lt;0.05</td>
<td>0.53</td>
</tr>
<tr>
<td>Serum bone alkaline phosphatase and serum IGF-I</td>
<td>0.42</td>
<td>&lt;0.05</td>
<td>0.48</td>
</tr>
<tr>
<td>Serum osteocalcin and serum IGF-I</td>
<td>—</td>
<td>NS</td>
<td>—</td>
</tr>
</tbody>
</table>

*Simple correlation analysis between the outcome variables (calcium absorption, urinary calcium, or each marker of bone turnover) and the serum hormones 1,25-dihydroxyvitamin D [1,25(OH)2 D], parathyroid hormone (PTH), and insulin-like growth factor I (IGF-I). NTx, N-telopeptides of type I collagen; EP, early pregnancy (10–12 wk); LP, late pregnancy (34–36 wk); EL, early lactation (7–8 wk).*
However, the hormonal regulators of bone turnover in these states are still unclear (1–3). Our study provides evidence that IGF-I contributes to the regulation of bone turnover during pregnancy and lactation. Naylor et al (27) found that changes in serum IGF-I during pregnancy were related to changes in urinary NTX and in serum propeptides of type I procollagen. In our study, a positive association was found between serum IGF-I and markers of bone turnover (urinary NTX and plasma bone-specific alkaline phosphatase) during pregnancy and with serum osteocalcin during lactation. IGF-I is known to increase osteoblastic cell recruitment and bone matrix deposition (20); therefore, it may regulate trabecular bone metabolism during pregnancy and lactation.

In conclusion, the results of this longitudinal study of Brazilian women during pregnancy and lactation, taken together with the results of Ritchie et al (5), provide evidence that the homeostatic mechanisms involved in providing calcium during pregnancy and lactation are probably influenced by maternal calcium intake. In addition to \( 1,25(\text{OH})_2\text{D} \), IGF-I and PTH appear to play major roles in calcium homeostasis during pregnancy and lactation.

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CLVZ was responsible for the clinical study, data collection, and data analysis and assisted with the writing of the manuscript. CMD was responsible for the supervision of the study and was involved in the study design, data analysis, and writing of the manuscript. LWR was involved in the management of the study, laboratory analysis, and revision of the manuscript. SAA was involved in the measurement of calcium absorption and revision of the manuscript. EMS was responsible for the IGF-I analyses and was involved in the discussion of the results. JCK was responsible for the conception and funding of the study and was involved in the study design and writing of the manuscript. None of the authors had a personal-interest affiliation with the supporter of this research.

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