Pregnancy and Lactation Affect Markers of Calcium and Bone Metabolism Differently in Adolescent and Adult Women with Low Calcium Intakes

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ABSTRACT  Physiological adaptation to the high calcium demand during pregnancy and lactation may be different in adolescents than in adults, particularly at low calcium intake. The aim of this cross-sectional study was to compare biochemical markers of calcium and bone metabolism between adolescent (14–19 y) and adult (21–35 y) women with calcium intake ~500 mg/d, in three different physiological states, i.e., control (nonpregnant, nonlactating; NPNL), pregnant and lactating. Markers of calcium metabolism [serum Ca, P and intact parathyroid hormone (iPTH); urinary Ca and P] and of bone turnover [urinary deoxypyridinolone (D-Pyr) and plasma bone alkaline phosphatase (BAP)] were measured in NPNL (adolescents, n = 12 and adults, n = 25), pregnant (adolescents, n = 30 and adults, n = 36) and lactating (adolescents, n = 19 and adults, n = 26) women. In the NPNL women, iPTH, D-Pyr and BAP were higher (P < 0.001) and of bone turnover [urinary deoxypyridinolone (D-Pyr) and plasma bone alkaline phosphatase (BAP)] were measured in NPNL (adolescents, n = 12 and adults, n = 25), pregnant (adolescents, n = 30 and adults, n = 36) and lactating (adolescents, n = 19 and adults, n = 26) women. In the NPNL women, iPTH, D-Pyr and BAP were higher (P < 0.001) and bone Ca was lower (P < 0.001) in adolescents than in adults. Serum iPTH was higher (P < 0.001) and urinary Ca was lower (P < 0.01) in adolescents than in adults also in pregnancy and lactation. Compared with NPNL women, serum Ca decreased (P < 0.001) with pregnancy in adolescents but not in adults. The increase in D-Pyr with pregnancy and lactation was pronounced in adults (~1100% in adolescents, suggesting that adolescents retain more calcium at skeletal maturity (1,2). The achievement of optimal peak bone mass is dependent on the rapid rate of bone accretion, mainly during the adolescence growth spurt, and on genetic and environmental factors including diet composition and calcium intake (1,3). However, in many developing countries including Brazil, calcium intake of adolescents is frequently lower than recommendations (4). In this situation, meeting calcium demands depends on the capacity for physiological adaptations in calcium metabolism.

There are few studies comparing calcium metabolism between adolescent and adult females and no studies evaluating possible differences in women with habitually low calcium intake. Kinetic and metabolic studies have shown that adolescent women absorb more calcium from the diet, excrete less calcium into urine and feces, have higher bone turnover rates and higher daily net calcium retention than do adults with similar high calcium intakes (2,3,5). Weaver et al. (2) also reported a significant relationship between urinary calcium excretion and net calcium absorption in adults but not in adolescents, suggesting that adolescents retain more calcium at higher intakes (>1300 mg/d), whereas in adults, the excess calcium absorbed is excreted into the urine. This is consistent with the higher calcium demand during adolescence.

Pregnancy and lactation are also periods of high calcium demands for fetal skeletal growth and milk production met mainly by physiological adjustments (6). During pregnancy, the major physiological adaptations to meet fetal needs include increased calcium intestinal absorption and increased rate of bone turnover (6–8). During lactation, there is a contribution of renal calcium conservation, but a temporary demineralization of the skeleton is the main mechanism to meet calcium requirements even when dietary calcium exceeds the recommended intake (6,8,9). These adjustments have been investigated mainly in adult women and are similar in women with a wide range of calcium intakes (10–13). Less is known about calcium homeostasis in pregnancy and lactation of adolescent women, particularly those with habitually low calcium intakes.

KEY WORDS: ● calcium ● adolescence ● pregnancy ● lactation ● bone turnover
The physiologic adaptation to pregnancy and lactation in adolescents may be different from that in adults, particularly at low calcium intake. Chan and collaborators (14) found a substantial reduction (8–10%) of bone mineral content during lactation in adolescent mothers consuming 900 mg Ca/d but no significant reduction in bone mineral in lactating adolescents consuming >1600 mg Ca/d (15). This suggests that pregnancy and lactation in adolescent women may increase susceptibility to bone mineral loss, especially when dietary calcium is low.

The purpose of this study was to compare biochemical markers of calcium and bone metabolism between adolescent and adult women with customary low calcium intakes in three different physiologic states, i.e., control, pregnancy and lactation.

**Subjects and Methods**

**Subjects and sample collection.** Pregnant (n = 66) and lactating (n = 45) women, recruited from a public prenatal clinic in Rio de Janeiro (Maternidade Escola/UFRJ), participated in the study after giving informed consent. Nonpregnant nonlactating (n = 37; NPNL) women also participated in the study as a control group and were recruited among students at UFRJ. The experimental protocol of the study was approved by the Ethical Committee of Maternidade Escola/UFRJ.

All women were apparently healthy, nonsmokers and had no history of bone or renal disorders affecting calcium metabolism. Pregnant women were at 28–39 wk of gestation; lactating women were at 6–20 wk postpartum, were exclusively breast-feeding, and gave birth to healthy infants. All NPNL women were nulliparous; pregnant and lactating women were primiparous and had no complications during pregnancy and/or lactation.

The NPNL women studied included 25 adults and 12 adolescents; the pregnant women, 36 adults and 30 adolescents; and the lactating women, 26 adults and 19 adolescents. Adults were 21–35 y of age and adolescents, 14–19 y of age. Within each adolescent group, results were similar between women 14–17 y old (NPNL, n = 6; pregnant, n = 19; lactating, n = 12) compared with 18–19 y old (NPNL, n = 6; pregnant, n = 11; lactating, n = 7) (data not shown). Therefore, results in adolescents are presented as a combined age group.

Habitual calcium intake of the women was assessed by three 24-h dietary recall questionnaires. Dietary nutrient analysis was done using the program “The Food Processor” (ESHA Research, Salem, OR) with the database adapted to Brazilian foods based on published information (16).

Morning blood (10 mL) and urine (50 mL) samples were collected from fasting woman between 0800 and 1000 h. Immediately after being drawn, 3 mL of whole blood was transferred into tubes with heparin for separation of plasma; the remaining blood was used for separation of serum. Aliquots of serum, plasma and urine, both acidified with HCl (final concentration 0.01 mol/L) and nonacidified, were stored at −20°C until analyzed.

**Laboratory analysis.** All materials used for sample collection, storage and analysis were either disposable or previously soaked overnight in dilute nitric acid (1:4) and carefully rinsed with deionized water.

Calcium in serum and urine was determined by complexing with methyl thymol blue; phosphorus in serum and urine by the method of Fiske-Subbarow; serum albumin with bromocresol green, and urinary creatinine by the Jaffe reaction, as previously described (17). Serum intact parathyroid hormone (iPTH) was measured by a two-site immunoradiometric assay (DSL, Webster, TX). Plasma activity of bone-specific alkaline phosphatase (BAP) was measured according to Farley et al. (18). Deoxypyridinoline (D-Pyr) crosslinks were determined in nonacidified urine by a competitive immunoassay (Metra Biosystems, Mountain View, CA). Urinary indices were expressed as creatinine ratios. Urinary creatinine excretion is similar in adults and adolescents ≥14 y old (19).

**Statistical analysis.** Data were analyzed using ANOVA followed by Tukey’s test for normally distributed variables, and using Kruskal-Wallis test followed by Dunn’s test for nonnormally distributed variables (iPTH, urinary calcium, urinary D-Pyr, and BAP). Interactions between physiologic state (NPNL, pregnant and lactating) and age group (adolescent and adult) were evaluated by two-way ANOVA using log-transformed values for nonnormally distributed variables. Differences with P < 0.05 were considered significant. The statistical analyses were performed using Statgraphics Version 7 for DOS (Manugistics, Cambridge, MA).

**RESULTS**

General characteristics of the women studied are shown in Table 1. The NPNL, pregnant and lactating women did not differ in age for either adults or adolescents. Adult and adolescent women had similar calcium intakes corresponding, on average, to 49 and 36% of recommended intakes in the U.S. and Canada for adults and adolescents, respectively (20).

In the adults, serum calcium was lower in pregnant than in lactating (P < 0.05) women; serum albumin was lower and the serum calcium:albumin ratio was higher in pregnant women than in both NPNL and lactating women (Table 2, P < 0.001). Serum phosphorus was higher (P < 0.05) in lactating women than in the other groups. Serum iPTH was lower in pregnant compared with lactating women (P < 0.001). Urinary calcium was lower in lactating than in NPNL and pregnant women (P < 0.001). Urinary phosphorus was lower in lactating than in pregnant women (P < 0.05). Pregnant and lactating women had higher (P < 0.001) urinary D-Pyr (133 and 125%, respectively) and higher (P < 0.001) plasma BAP (59 and 65%, respectively) compared with NPNL women.

In the adolescents, serum calcium and albumin were lower

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adult, nonlactating</th>
<th>Adult, lactating</th>
<th>Adolescent, nonlactating</th>
<th>Adolescent, lactating</th>
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<tr>
<td>n</td>
<td>25</td>
<td>36</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>Age, y</td>
<td>24.9 ± 3.7</td>
<td>26.1 ± 5.3</td>
<td>17.3 ± 1.14</td>
<td>26.2 ± 4.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.8 ± 2.7</td>
<td>23.3 ± 4.52</td>
<td>20.3 ± 3.7</td>
<td>22.8 ± 2.8</td>
</tr>
<tr>
<td>Dietary calcium intake, mg/d</td>
<td>489 ± 164</td>
<td>398 ± 124</td>
<td>458 ± 151</td>
<td>593 ± 126</td>
</tr>
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</table>

1 Values are means ± sd. a Significantly different from the corresponding adult women (P < 0.05).
2 Based on prepregnancy weight.
TABLE 2

Biochemical indices of calcium and bone metabolism in nonpregnant, nonlactating (NPNL), pregnant and lactating adolescent and adult women

<table>
<thead>
<tr>
<th></th>
<th>NPNL</th>
<th>Pregnant</th>
<th>Lactating</th>
<th>P-values</th>
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<tr>
<td></td>
<td>Adult</td>
<td>Adolescent</td>
<td>Adult</td>
<td>Adolescent</td>
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<tr>
<td>n</td>
<td>25</td>
<td>12</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>Serum calcium, mmol/L</td>
<td>2.21 ± 0.15&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.29 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.02 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>48 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ± 3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>37 ± 3&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calcium:albumin, μmol/g</td>
<td>46.4 ± 4.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.4 ± 5.7&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>57.6 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.1 ± 4.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum phosphorus, mmol/L</td>
<td>1.12 ± 0.15&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.23 ± 0.19&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.08 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.21 ± 0.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary calcium, mmol/mmol creatinine</td>
<td>1.2 (0.2-2.8)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>2.1 (1.1-3.6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9 (0.6-3.2)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.9 (0.7-5.4)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Urinary sodium, mmol/mmol creatinine</td>
<td>0.21 (0.06-0.59)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.12 (0.07-0.22)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23 (0.07-1.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 (0.05-0.70)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary calcium: sodium, mmol/mmol</td>
<td>9.0 (2.9-29.4)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.8 (1.7-14.4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.1 (8.0-40.4)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.7 (3.5-45.8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary phosphorus, mmol/mmol creatinine</td>
<td>21 (6-55)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19 (9-41)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>15 (5-55)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16 (3-46)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary creatinine, mmol/mmol creatinine</td>
<td>1.75 ± 0.84&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.02 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.05 ± 0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Deoxypyridinoline, nmol/mmol creatinine</td>
<td>5.2 (1.0-11.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.9 (3.6-11.3)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>12.2 (4.3-11.3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.3 (2.5-20.5)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma bone alkaline phosphatase, uL plasma</td>
<td>9.7 (1.7-16.2)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.5 (11.7-44.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.4 (8.9-35.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.4 (6.9-35.8)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>1</sup> Values are means ± so or medians (minimum-maximum). Values with different superscript letters in a row differ, P < 0.05. NS, nonsignificant, P ≥ 0.05.

...in pregnant compared with NPNL and lactating women (P < 0.001) but the serum calcium:albumin ratio did not differ among groups. Serum phosphorus also did not differ among adolescent groups but serum iPTH was lower in pregnant than in NPNL (P < 0.001) and lactating (P < 0.01) women. Urinary calcium was lower in lactating than in NPNL and pregnant women (P < 0.001), as observed in adult women; moreover, urinary calcium was higher in pregnant than in NPNL women (P < 0.001). Urinary phosphorus did not differ among groups. Compared with NPNL women, urinary D-Pyr was lower (P < 0.001) in pregnant and in lactating women as observed in adults but increases were of a smaller magnitude (4 and 24%, respectively). Unlike in adults, plasma BAP was lower (P < 0.001) in pregnant compared with NPNL and lactating women (13 and 35%, respectively).

Biochemical indices were compared between adolescent and adult women within each physiologic state. In NPNL women, serum iPTH, urinary D-Pyr, and BAP were higher (P < 0.001) in adolescent than in adult women (75, 71 and 91%, respectively), whereas urinary calcium was 43% lower (P < 0.001). In the pregnant women, serum calcium, urinary calcium, urinary phosphorus and urinary D-Pyr were lower (P < 0.01) in adolescents compared with adults (6, 22, 27 and 24%, respectively), whereas serum iPTH was 110% higher (P < 0.001). In the lactating women, serum iPTH and BAP were higher (P < 0.001) in adolescents than in adults (63 and 39%, respectively), whereas urinary calcium was 57% lower (P < 0.001).

Significant interactions between physiologic state (NPNL, pregnant and lactating) and age group (adult and adolescent) were observed for serum calcium (P < 0.001), urinary D-Pyr (P = 0.013), and plasma BAP (P < 0.001). The decrease in serum calcium associated with pregnancy (physiologic state as main effect, P < 0.001) occurred mainly in adolescents. The increase in urinary D-Pyr with pregnancy and lactation (P < 0.001) was very pronounced in adults (−130%) but less in adolescents (−25%). BAP increased with pregnancy and lactation (−60%) in adults but decreased with pregnancy (−13%) and did not change with lactation in adolescents.

**DISCUSSION**

Adolescents typically have a higher rate of bone turnover than adults (2) and may be less sensitive to further increase in bone turnover because of pregnancy and lactation. However a low calcium intake in adolescent women may limit the max-
imal rates of bone turnover, possibly affecting its response to pregnancy and lactation. Our study is the first to compare biochemical markers of calcium and bone metabolism between adolescent and adult women with similar low calcium intakes, in pregnancy and lactation, compared with nonpregnant nonlactating controls. Mean calcium intake in the women of our study, 400–500 mg/d, appears to be common in Brazil (4,21).

Comparisons between NPNL adolescent and adult women. Similar levels of serum calcium and serum PTH have been observed in adolescent and adult women with calcium intakes >1000 mg/d (2). In the present study, serum calcium was similar, but serum iPTH was higher in adolescent compared with adult women, possibly as a response to their low calcium intake. In fact, serum PTH has been found to be negatively related with dietary calcium in adolescents (22). A low calcium intake (~400 mg/d) appeared to induce a compensatory hypersecretion of PTH in adolescent women (22) and a similar response may be occurring in our study.

Studies have shown that adolescent women with adequate calcium intake excrete approximately half the amount of calcium in urine as excreted by adult women (2,3). A similar reduction was observed in our study in women with low calcium intakes. Fasting urinary calcium in adolescents was approximately half that in adults. In fact, urinary calcium excretion in adolescence represents obligatory renal calcium losses, independent of calcium intake, whereas in adults, this excretion reflects dietary calcium intake (23).

Markers of bone formation and resorption were found to be two- to fivefold higher in adolescent compared with adult women with adequate calcium intakes (5), whereas in our study of women with low calcium intakes, differences in these markers between adolescent and adult women were less pronounced (less than twofold). Therefore, the typical increase of bone turnover in adolescence appears to be limited when calcium intake is low.

Comparisons between pregnant adolescent and adult women. Serum calcium and serum iPTH decreased with pregnancy in adolescents but not in adults. Similar to NPNL women, adolescents had higher levels of serum iPTH than adults during pregnancy, but also lower serum calcium. PTH levels do not usually change with pregnancy (24) as observed in the adult women in our study, or are slightly decreased (24) as observed in the adolescent women. Pregnancy is typically associated with a fall in serum total calcium as a result of decreased serum albumin concentration because of hemodilution (24). In our study, this fall was more evident in adolescents. Although we did not measure serum ionized calcium, it is possible that adolescent pregnant women have reduced serum ionized calcium levels compared with adult pregnant women because no difference in albumin levels was found between adults and adolescents. This may reflect a more intense calcium tissue uptake, both for fetal growth and maternal bone accretion in adolescent pregnant women.

Pregnancy is usually associated with an increase in urinary calcium excretion (10) due primarily to increased glomerular filtration rate and increased intestinal calcium absorption (7,8). In the present study, an increase in fasting urinary calcium associated with pregnancy occurred only in the adolescent women. However, pregnant adolescents had lower fasting urinary calcium than pregnant adults although the difference was less pronounced in pregnant than in NPNL women (22 vs. 43%). Our results suggest that, at least when calcium intake is low, the physiologic mechanism operating in adolescence that efficiently conserves renal calcium for bone accumulation remains active during pregnancy although to a lesser extent.

Pregnancy has been found to substantially increase bone turnover in adult women both with adequate (10,13) and low (17) calcium intakes, as seen in the adult women in the present study. In the adolescents, however, pregnancy seemed to increase bone resorption to a lesser extent than in adults and to decrease bone formation unlike in adults. Moreover, bone formation, as indicated by BAP, did not differ between adolescent and adult pregnant women. Therefore, the expected higher bone formation rate in adolescents than in adults was not observed during pregnancy. These results suggest that skeletal growth of adolescents may be suppressed during pregnancy. In contrast to NPNL women, bone resorption in pregnant women, as indicated by urinary D-Pyr, was lower (and not higher) in adolescents than in adults. This may be a protective adaptation in pregnant adolescents, at least when calcium intake is low, that decreases the risk of bone loss associated with increased bone turnover during pregnancy.

Comparisons between lactating adolescent and adult women. Serum calcium, albumin, and iPTH did not differ between lactating and NPNL women either in adults or adolescents, as expected in normal lactation (24). However, serum iPTH was higher in lactating adolescents than in lactating adults, as observed in the NPNL and pregnant groups, probably as an adaptive response to low calcium intake associated with the high calcium demand in adolescence.

Urinary calcium excretion is typically reduced in adult lactating women with both high (13) and low (25,26) calcium intakes. In the present study, fasting urinary calcium was lower in the lactating than in NPNL or lactating pregnant women, both in adults and adolescents. However, urinary calcium was substantially lower in lactating adolescents compared with lactating adults, as observed in the NPNL women. The typical renal calcium conservation in lactation appears to be very efficient in lactating adolescents with low calcium intakes.

Biochemical markers of bone resorption and formation are substantially elevated during lactation irrespective of calcium intake (6), as seen in the adult women in the present study. However, in the adolescents, urinary D-Pyr was only slightly higher in lactating compared with NPNL women and BAP did not differ between these groups. Although small differences in bone biomarkers should be interpreted with caution, our results suggest that the already high bone turnover in adolescents is little affected by lactation. Similar to NPNL women, BAP in the lactating women was higher in adolescents compared with adults, although the increase was less pronounced. This suggests that during lactation, bone formation is gradually reestablished in adolescents.

Pregnancy and lactation appeared to affect markers of bone turnover in adolescent and adult women with low calcium intakes differently. The increased bone resorption associated with pregnancy and lactation was less pronounced in the adolescents, possibly as a protective mechanism against excessive bone loss. Pregnancy, but not lactation, seemed to impair bone formation in the adolescents. During lactation, adolescents may be partially catching up on skeletal growth that ceased or slowed down during pregnancy.

ACKNOWLEDGMENTS

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LITERATURE CITED


